Faculty of Agriculture and Forestry
Department of Food and Nutrition
Doctoral Programme in Food Chain and Health
University of Helsinki, Finland

VTT Technical Research Centre of Finland Ltd

EKT Series 1987

DRY FRACTIONATION AND FUNCTIONALISATION OF CEREAL SIDE STREAMS FOR THEIR IMPROVED FOOD APPLICABILITY

Pia Silventoinen

DOCTORAL DISSERTATION

To be presented for public examination with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, in lecture hall B3, Forest Sciences Building, Latokartanonkaari 7, on the 3rd of March 2021, at 12 noon.

Helsinki 2021

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ISBN 978-951-51-7113-9 (paperback)
ISBN 978-951-51-7114-6 (PDF; https://ethesis.helsinki.fi/)
ISSN 0355-1180

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ABSTRACT

The agro-food industry generates annually substantial amounts of side streams, resulting in the loss of high-quality protein and dietary fibre, whereas their incorporation into the food chain would positively contribute to resource sufficiency and healthier diets. However, plant-based ingredients, especially proteins, typically deliver limited performance in certain food applications, such as beverages and spoonable products, when compared with their animal-based counterparts. Therefore, fractionation and functionalisation techniques are investigated and applied to improve the applicability of the plant-origin ingredients in a wider range of food matrices where they can offer alternatives to animal-based ingredients. Dry fractionation provides a sustainable and gentle processing technology, which allows the production of multicomponent hybrid-ingredients, enriched in protein but also containing considerable amounts of dietary fibre or starch, depending on the raw material. The aim of the current work was to investigate the use of dry fractionation, more specifically, dry milling and air classification, for increasing the protein content of cereal side streams, namely, wheat, rice and rye brans, and the barley endosperm fraction. In addition, the objective was to understand the factors affecting the technological functionality and applicability of the protein-enriched ingredients in the relevant food matrices. To facilitate a more efficient fractionation, pre-treatments, including defatting with supercritical carbon dioxide (SC-CO_2) for rice bran, moisture removal for wheat and rye brans and mixing with a flow aid for the barley endosperm fraction, were elucidated. The technological functionality of the protein-enriched fractions was examined, and bioprocessing and physical processing approaches for improving the ingredient applicability in high-moisture food systems were investigated with rice and barley fractions.

This study revealed that the fat removal, drying and use of flowability aids were effective in enhancing dry fractionation by improving the processability, particle size reduction and dispersability of rice bran, wheat and rye brans, and the barley endosperm fraction, respectively. Pin disc milling and air classification of a SC-CO_2-extracted rice bran increased the protein content from 18.5 to 25.7% with 38.0% protein separation efficiency (PSE). Alternatively, a two-step air classification of the defatted rice bran allowed to reach a slightly higher protein content (27.4%) with lower PSE (20.2%) compared with the one-step air classification approach. Air classification of the dried and pin disc-milled wheat and rye brans increased the protein content from 16.4 and 14.7%, respectively, to 30.9 and 30.7%, with PSE of 18.0 and 26.9%. Additionally, soluble-to-insoluble dietary fibre ratios were increased and phytic acid was considerably enriched in bran fractionations. The maximum protein content reached by air classification from the barley endosperm fraction, initially containing 80.0% starch and 8.3% protein, was

28.3% with 21.7% PSE, while reaching a lower protein enrichment level of 22.3% allowed obtaining PSE of 59.4%.

The protein-enriched fractions, especially those from rice and wheat, exhibited higher protein solubility than the raw material brans, presumably due to the enrichment of albumin and globulin proteins from the aleurone during air classification, which was also indicated by an altered protein profile and the co-enrichment of phytic acid. When the ultra-fine milling of wheat and rye brans was explored as an alternative to fractionation, the formation of damaged starch and lowered protein solubility were observed. The protein-enriched brans and the ultra-finely milled brans both showed improved dispersion stabilities, whereas pasting viscosities, and water and oil binding capacities were lower for the hybrid ingredients compared with the pin disc-milled raw materials. The protein-enriched fraction from barley, on the other hand, exhibited low protein solubility and limited techno-functional properties.

The applicability of the protein-enriched fractions in high-moisture food model systems was tested after ingredient modifications via enzyme treatment, ultrasonication and pH shifting. Phytase treatment of the protein-enriched rice bran fraction improved the behaviour of the ingredient in heat-induced gelation, especially under alkaline conditions. For the protein-enriched barley fraction, ultrasound treatment with or without pH shifting reduced particle size; improved colloidal stability at pH 3, 7 and 9; and increased protein solubility, especially at pH 9.

To conclude, dry fractionation of cereal side streams allowed protein enrichment with a concurrent increase in the soluble-to-insoluble dietary fibre ratios of the brans and considerable reduction in the starch content of the barley endosperm fraction. Additionally, this thesis demonstrated for the first time that cereal side stream-derived, protein-enriched hybrid ingredients exhibit improved technological functionalities that can be further enhanced via enzymatic or physical processes that affect, for example, their gelation and dispersion stability. The bioprocessed protein-enriched rice bran fraction could find potential use as a raw material in spoonable food products delivering a good amount of protein and dietary fibre and allowing the use of the nutritional claim that the food is a ‘source of fibre’. The ultrasound-treated barley protein ingredients, on the other hand, should be further studied in the manufacturing of plant-based milk substitutes. In general, these improved ingredient properties suggest the possibility of developing novel side stream-based food ingredients with increased nutritional and technological qualities that simultaneously contribute positively to raw material resource sufficiency.

ACKNOWLEDGEMENTS

This study was carried out at VTT Technical Research Centre of Finland Ltd during the years 2016–2020. The research leading to this work received funding from the Bio-based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme under grant agreement No. 668953−PROMINENT, and from Nordic Innovation under grant number P14046–FUNPRO. The Finnish Food and Drink Industries' Federation and Doctoral Programme in Food Chain and Health kindly provided funding for participating in conferences and performing some of the experiments. All financial support is highly appreciated. Südzucker AG, Altia Corporation and Fazer Mills are acknowledged for providing raw materials for the work.

I wish to express my most sincere gratitudes to my supervisors Docent Emilia Nordlund, Dr. Dilek Ercili-Cura and Research Professor Nesli Sözer, for their invaluable support and guidance throughout this work. I am deeply grateful to my wonderful team leader Emilia, who has supervised me ever since my master's thesis work, for her excellent scientific insights and for being always available for discussions despite her overflowing schedule. Her enthusiasm towards improving the food system bite by bite is something truly admirable. I am thankful to Nesli for all her inspiring ideas and input to my work, and for entrusting me with various demanding tasks within this PhD and other projects that have allowed me to develop into a more mature researcher. I warmly thank Dilek for her constructive guidance, caring and mentoring, and sharing similar thoughts in various aspects, which has led to many long discussions especially during the mid-part of my PhD. I truly appreciate the way in which you value the power of research and essence of science.

I wish to thank Senior Advisor Kaisa Poutanen for guiding me especially in the beginning of my research career and acting as a member of my thesis advisory committee. Her enthusiasm in cereal science and research is an inspiration to us all. Associate professor Kati Katina is acknowledged for her guidance during the years of my doctoral studies and for acting as a member of the thesis advisory committee.

I am sincerely thankful to all my co-authors for their invaluable contributions and advice. I wish to thank my colleague Dr. Ulla Holopainen-Mantila for her assistance in interpreting the microscopy analyses and for the various fruitful discussions. I thank Dr. Katariina Rommi for her excellent guidance during the first steps of my research career and Dr. Mika Sipponen for the valuable collaboration related to Publication III. I am thankful to my colleague Anni Kortekangas for the collaboration, friendship and insightful discussions related to gelation ever since her master's thesis in which she carried out major parts of the experimental work of Publication IV under my supervision. I am thankful to my pre-examiners Dr. Cécile Barron and Professor Milena Corredig for their

valuable feedback and suggestions during the preliminary examination of this thesis.

I am sincerely thankful for VTT for providing the world-class research facilities and supporting the work financially but also for allowing me to learn key elements about project management, research collaboration and customer relationships along the path to PhD. I deeply thank all the former and current members of our Food Solutions team for creating an inspiring, warm and supportive working environment each day. I am grateful to VTT's excellent and helpful technical staff for all their assistance and contributions to the experimental work. Especially, I wish to thank Riitta Pasanen, Leila Kostamo, Eero Mattila, Anna-Liisa Ruskeepää, Tarja Wikström, Tytti Salminen and Niklas Fred for their never-ending helpful attitude and all the cheerful moments in the lab. Eva Fredriksson-Haramo is acknowledged for being always available to help in any daily issues. Special thanks go to Anni Nisov – I am extremely thankful for both your friendship and scientific support and I will always cherish our fascinating discussions related to everything between protein conformation and livingroom decoration. I wish to thank my roommate Natalia Rosa-Sibakov for cheerful discussions and all the advice throughout these years. I wish to thank Iina Jokinen and Anna-Maria Sneck who enabled me to deep dive also into the world of oats during supervision of their master's thesis works. I want to thank Markus Nikinmaa, Alex Calton, Outi Nivala, Heikki Aisala, Martina Lille, Kaisu Honkapää, Eeva Rantala, and many others for the recovering lunch sessions, virtual coffee breaks and inspiring discussions.

I extend my heartfelt thanks to my family and friends for all their encouragement, support and interest towards this work. I am grateful to my mother for her love and care throughout my life. I thank Matilda, Jaana, Kari, Laura and Ed for all the joyful moments and encouragement. I am grateful for Susanne, Saara, Sari and many other dear friends for all the happiness, support and enlightenment that you bring to my days.

Finally, I owe my dearest thanks to my beloved fiancé, Antti, for sharing all the possible micro- and macro-scale joys and griefs in life. Your empowering love, enlightening support and our cheerful moments and adventures are priceless to me!

Espoo, January 2021

Pia Silventoinen

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Silventoinen P, Rommi K, Holopainen-Mantila U, Poutanen K, Nordlund E. 2019. Biochemical and techno-functional properties of protein- and fibre-rich hybrid ingredients produced by dry fractionation from rice bran. Food Bioprocess Technol. 12(9):1487–1499.

II Silventoinen P, Kortekangas A, Ercili-Cura D, Nordlund E. 2021. Impact of ultra-fine milling and air classification on biochemical and techno-functional characteristics of wheat and rye bran. Food Res. Int. 139:109971.

III Silventoinen P, Sipponen MH, Holopainen-Mantila U, Poutanen K, Sozer N. 2018. Use of air classification technology to produce protein-enriched barley ingredients. J. Food Eng. 222:169–177.

IV Kortekangas A, Silventoinen P, Nordlund E, Ercili-Cura D. 2020. Phytase treatment of a protein-enriched rice bran fraction improves heat-induced gelation properties at alkaline conditions. Food Hydrocoll. 105:105787.

V Silventoinen P, Sozer N. 2020. Impact of ultrasound treatment and pH-shifting on physicochemical properties of protein-enriched barley fraction and barley protein isolate. Foods 9:1055.

The publications are referred to in the text by their roman numerals.

AUTHOR'S CONTRIBUTIONS

I Pia Silventoinen participated in designing the study under the supervision of Katariina Rommi, Emilia Nordlund and Kaisa Poutanen. She was responsible of the experimental work and conducted the dry fractionation experiments, protein analytics, particle size determinations, analysis of the techno-functional properties and statistical analysis. She had the main responsibility for the interpretation of the results and writing the publication with her co-authors. Ulla Holopainen-Mantila had the main responsibility for the microscopy analyses.

II Pia Silventoinen designed and coordinated the work under the supervision of Dilek Ercili-Cura and Emilia Nordlund. She was responsible for the experimental work and also had the main responsibility for the data analysis, statistical analysis and interpretation of the results. She was responsible for writing the publication together with all the co-authors.

III Pia Silventoinen participated in designing the research together with Mika Sipponen under the supervision of Nesli Sözer and Kaisa Poutanen, conducted part of the dry fractionation experiments and was responsible for the characterisation of the ingredients. She had the main responsibility for the data analysis, statistical analysis, interpretation of the results and writing the publication together with her co-authors. Ulla Holopainen-Mantila had the main responsibility for the microscopy analyses.

IV Pia Silventoinen coordinated the work and designed it together with Anni Kortekangas under the supervision of Dilek Ercili-Cura and Emilia Nordlund, conducted dry fractionation experiments for ingredient preparation, supervised the master's thesis student/research trainee, Anni Kortekangas, in the execution of the experimental work and was partially responsible for the microscopy analysis. She and Anni Kortekangas had the main responsibility (equal authorship) for the data analysis, interpretation of the results and writing the publication together with the other co-authors.

V Pia Silventoinen had the main responsibility for designing the experimental set-up under the supervision of Nesli Sözer. She planned the dry fractionation experiments for the industrial scale, designed the protein isolation experiments and was responsible for the ultrasound treatments. Moreover, she had the main responsibility for the data analysis, the interpretation of the results and writing the publication together with Nesli Sözer.

ABBREVIATIONS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Collaboration International
ANS	1-anilino-8-naphthalene sulfonate
DF	dietary fibre
dm	dry matter
G'	storage modulus
G''	loss modulus
IDF	insoluble dietary fibre
PSE	protein separation efficiency
RVA	Rapid Visco Analyser
SC-CO_2	supercritical carbon dioxide
SDF	soluble dietary fibre
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
tan δ	loss tangent
WBC	water binding capacity (of a flour)
WHC	water holding capacity (of a gel)

1 INTRODUCTION

Due to major global challenges, including climate change and population growth, the agro-food industry is urged to take actions towards more sustainable approaches for food production. The transformation of the global food system into more plant-based direction is needed from both resource sufficiency and human health perspectives. Dietary patterns have a major role in food security, and the sustainability and environmental impact of foods. The exploitation of proteins derived from plants and industrial side streams are currently increasingly studied due to the verified negative impacts of meat and dairy production on the environment (Poore and Nemecek 2018; Springmann et al. 2018). In addition, plant-based diets are also considered healthier (Willett et al. 2019).

The industrial milling of cereal grains into refined flours for food use has been applied for centuries. However, the milling processes always result in the production of side streams that are not fully exploited for food but rather applied for feed or in energy production. The milling of grains, that targets the production of white endosperm flour, yields vast amounts of underutilised bran and germ fractions which are rich in nutritionally valuable components, such as protein and dietary fibre (DF) (Delcour and Hoseney 2010). In addition to milling, other cereal ingredient, food and beverage production processes generate side streams. Valorisation of these cereal side streams as food ingredients would not only provide improved sustainability via resource sufficiency but also promote the utilisation of the full nutritional potential of cereal grains in human diets. Currently, feasible solutions for the valorisation of cereal side streams as appealing and functional food ingredients are still lacking.

The challenges regarding the utilisation of cereal side streams in food applications include the low level of component purity in the side streams and the inferior techno-functional properties of cereal proteins compared with their animal-based counterparts, resulting from the large molecular weight and low water solubility of the plant proteins. Hence, improving the performance of the target components in food applications necessitates fractionation and further functionalisation. However, ingredient purification aiming at the manufacture of concentrates or isolates is generally obtained at the expense of other favourable ingredient attributes. As illustrated in Figure 1, a higher level of purification may result in improved technological ingredient functionality, but the processing costs increase concurrently. On the contrary, the less refined ingredients contain more of the raw material macro- and micronutrients and fewer side streams are generated during their production. Further, less processing also allows retention of the native functionality of the ingredients that may be lost in isolation processes applying harsh treatment conditions. Indeed, instead of aiming at producing pure isolates by water-intensive

processes, it is equally important to focus on the design of multi-component, less-refined food ingredients, enriched in nutritionally favourable components. The wet extraction of cereal side stream proteins for use as added-value ingredients has been rather extensively studied, whereas only a little is known about the potential of dry fractionation in side stream valorisation. Dry fractionation refers to dry unit operations, such as milling and air classification, which target the modification or separation of components in dry materials as a result of physical forces acting on them. By dry fractionation, the under-utilised cereal side streams can be converted into multi-component food powders, also referred to as hybrid ingredients, enriched in protein and, for example, DF, thereby offering nutritionally and technologically valuable, new and resource-efficient food ingredients. However, the general limitations of plant-based ingredients, including, for example, poor protein solubility, low dispersion stability, the presence of antinutritional factors and taste challenges, apply to the dry-fractionated ingredients as well, and thus, further functionalisation via, for example, physical, biochemical or hydrothermal approaches may be required.

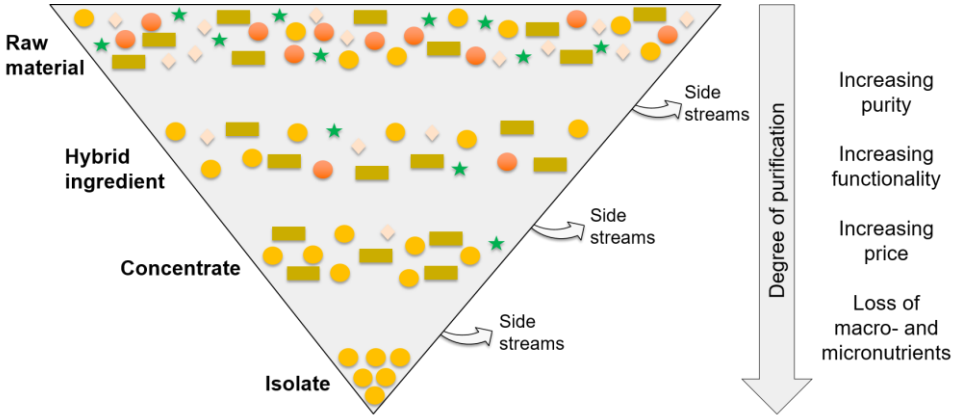

Figure 1. A schematic presentation of the impact of food ingredient purification on the properties of the ingredient.

The current work focuses on the valorisation of cereal side streams using dry fractionation alone or in combination with bioprocessing or physical processing, targeting their improved applicability in high-moisture food systems. In the literature review, the most important cereal side streams and their production processes are summarised. Additionally, dry processing approaches for cereal component fractionation and technological functionality and the functionalisation of cereal ingredients are covered. The literature part concentrates mainly on the cereal grains studied in this work (i.e. rice, wheat, rye and barley), but other cereal grains (for example, oats) are discussed when relevant. The experimental part of this thesis focuses on the development of suitable dry fractionation approaches for the cereal side streams deriving from rice, wheat, rye and barley. Moreover, the study elucidates the impact of both dry fractionation and selected bio- or physical processing on the techno-functional properties of the dry-fractionated cereal ingredients.

2 REVIEW OF THE LITERATURE

2.1 SIDE STREAMS IN CEREAL GRAIN PROCESSING

Cereal grains are staple foods for most of the world's population. Globally, maize has the largest annual production volume among the cereal grains and, in 2018, the production was 1 147.6 million tonnes (FAOSTAT 2018; Table 1). Rice ranks second with annual production of 782.0 million tonnes of paddy rice (FAOSTAT 2018). Wheat is the third most abundantly produced cereal grain with 734.0 million tonnes produced annually, whereas various coarse grains (e.g. barley, sorghum, millet, oats and rye) are produced in lesser quantities (FAOSTAT 2018). In addition to being consumed as food, use for feed and in biofuel production are important areas of cereal grain consumption. Milled rice is mainly utilised for food (81.0%) whereas only approximately 70% of wheat is used for human consumption (OECD/FAO 2020; Shiferaw et al. 2013). Even more noticeably lower amounts of maize (12.4%) and other coarse grains (27.9%) are consumed as food (OECD/FAO 2020).

Table 1. Annual global production and consumption quantities of the major cereal crops. Consumption is divided into food, feed and biofuel consumption and the values presented in parentheses account for the share of total consumption of each consumption category. Additionally, the amounts of brans are listed for the main crops that contribute significantly to global agricultural side stream generation.

	Production[a]	**Consumption[b]**				**Bran production**
	Mt	**Total Mt**	**As food Mt**	**As feed Mt**	**As biofuel Mt**	**Mt**
Maize	1 147.6	1 141.5	141.8 (12.4%)	675.1 (59.1%)	181.4 (15.9%)	20–23[e]
Wheat	734.0	747.4	511.5 (68.4%)	149.4 (20.0%)	9.2 (1.2%)	56–92[f]
Rice	782.0[c]	511.7[d]	414.2 (81.0%)	17.8 (3.5%)	0.0 (0.0%)	63–76[g]
Other coarse grains	266.1	282.6	78.9 (27.9%)	144.9 (51.3%)	9.1 (3.2%)	-
Barley	141.4	-	-	-	-	-
Sorghum	59.3	-	-	-	-	-
Millet	31.0	-	-	-	-	-
Oats	23.1	-	-	-	-	-
Rye	11.3	-	-	-	-	-

Mt: million tonnes.
[a] FAOSTAT (2018), data from 2018.
[b] OECD/FAO (2020), data expressed as average from 2017–2019 (estimation).
[c] expressed as paddy rice.
[d] expressed as milled rice.
[e] calculated based on the food use amount and pericarp (5–6% of the grain) and germ (9–10% of the grain) shares of the grain (Chaudhary et al. 2014; Delcour and Hoseney 2010).
[f] calculated based on the food use amount and bran share of the kernel (11–18%).
[g] Kahlon (2009).
- : not available / not relevant.

Cereal grain processing generates vast amounts of side streams from milling and biorefining industries. In addition to the low-valued fractions from the milling industry, such as husk and bran, cereal-derived side streams include brewer's spent grain (BSG) and malt sprouts from brewing, dried distiller's

grains with solubles (DDGS) from bioethanol production and protein-rich streams from the wet extraction of cereal starches, as reviewed by Galanakis (2018). Moreover, some of the first-step side streams, such as corn germ and rice bran, are commonly further processed by oil extraction steps, resulting in high-value oils and secondary side streams, such as defatted corn germ and defatted rice bran.

The most essential process for cereal grains that targets food manufacturing is dry milling, which results in the production of food ingredients, such as flours, flakes and brans. The various steps in dry milling are distinctive for each cereal species. Most cereal grains are processed using cleaning, sorting, dehulling (for the hulled grains) and dry milling steps, resulting in whole grains and, further, either polished or pearled grains, or fractionation-based refined flour, as reviewed by Delcour and Hoseney (2010). Due to the removal of the outer grain layers and germ that are high in protein, DF, vitamins, minerals and oils, the refined flour ingredient from the milling process is recognised as having lower nutritional value than the whole grain but is widely used owing to its superior techno-functional and sensory qualities. The side streams produced in the dry processing of cereal grains are discussed in the following Sections 2.1.1 and 2.1.2.

2.1.1 SIDE STREAMS DERIVING FROM REFINING CEREAL GRAINS INTO WHITE FLOUR

The dry milling of cereal grains includes different approaches to physically detach the grain structure. In general, the term dry milling accounts for all the unit operations involved in the transformation of unprocessed native grains into cereal-derived food ingredients (Figure 2). In these processes the aim is to separate the different botanical parts of the grains, such as the starchy endosperm, outer kernel layers and the germ, in order to produce refined ingredients. Depending on the grain type, the milling procedures vary and may result in, for example, dehulled grains, pearled or polished grains, grits, coarse semolina or fine flour (Delcour and Hoseney 2010).

In the first step of the milling process, all cereal grains are cleaned using several unit operations. Then, the grains containing hulls after harvesting (e.g. rice and barley) are dehulled, resulting in the first side-stream fraction of the grain milling process. In rice, the hulls are not fused with the outer grain layers and contribute to 20% of the whole grain (Evers and Millar 2002). In barley, the hull, accounting for 10–13% of the grain, is cemented to the outer pericarp layers. Therefore, it requires pearling in order to be removed and this usually results in the partial removal of the pericarp, seed coat and aleurone layers (Delcour and Hoseney 2010; Evers and Millar 2002). Thus, the side stream fraction from barley grain refining usually contains hull and also some outer bran layers. Cereal hulls are mainly composed of non-starch polysaccharides and ash, and the amount of protein is low (Delcour and Hoseney 2010). The main use for cereal hulls is currently found in direct energy production

(Galanakis 2018) but, for example, for rice hulls, the uses vary from soil amendments to feed and litter, and to bedding for poultry (Bodie et al. 2019).

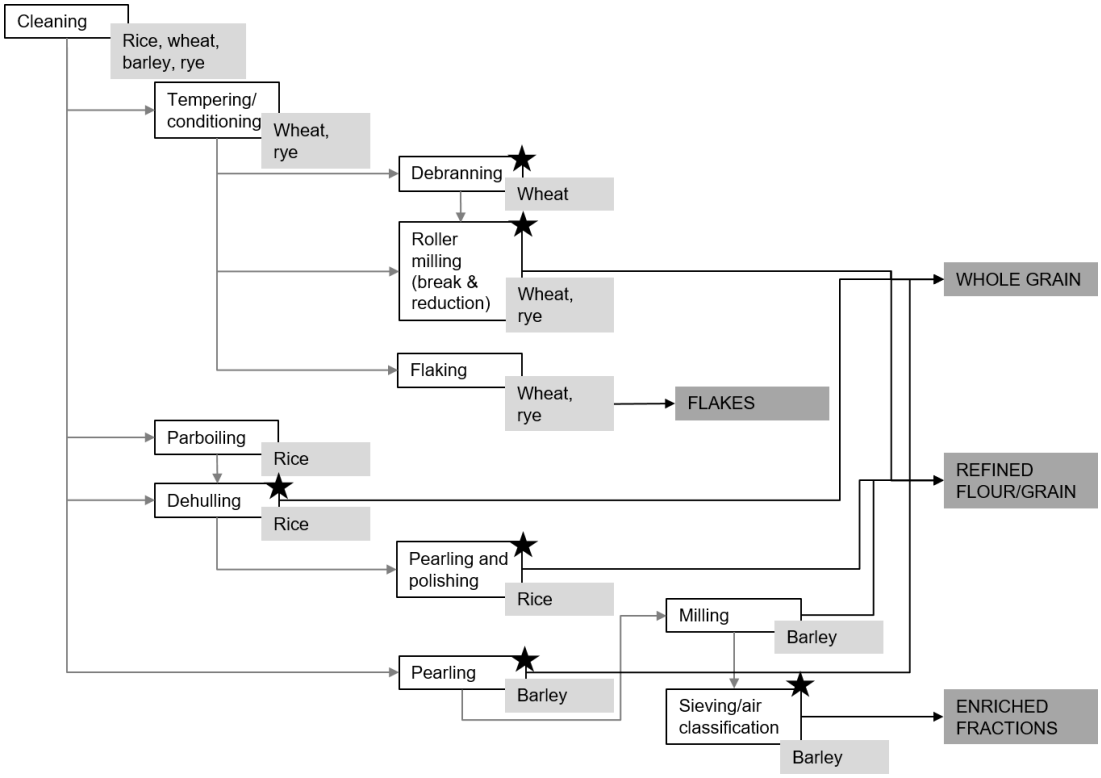

Figure 2. A simplified processing scheme illustrating the main unit operations in wheat, rice, barley and rye milling. The white-coloured boxes represent processing steps and the light grey boxes show the grains that are processed with each method. The dark grey boxes indicate the final products. The processes where side streams are generated are marked with black stars.

The dehulled grains, like brown rice and pearled barley grains, can be directly consumed as food or milled to obtain whole grain flour. However, the refined cereal ingredients are considered more valuable and, thus, the whole grains are generally further processed. The most distinctive step of cereal grain milling is related to the removal and separation of bran from the starchy endosperm, which yields the bran fraction composing of the outer grain layers (the pericarp, seed coat and nucellus) and often also the germ (Figure 3). For most cereal grains, the bran fraction also includes the endosperm-derived constituents, aleurone and subaleurone. Moreover, varying amounts of inner starchy endosperm regions may be present in the bran preparation. Table 2 lists the composition of the common cereal brans. As shown in Figure 2, the process for the removal of the outer grain layers from the starchy endosperm differs for each species.

Wheat and rye bran separation usually initiates with tempering and conditioning steps (i.e. the addition of water), which soften the starchy endosperm and toughen the bran, allowing to remove the the bran from the starchy endosperm as larger pieces during the milling process (Delcour and Hoseney 2010). For wheat and rye, bran separation takes place in a roller mill system in which the grain is crushed and sieved in multiple steps. Bran

represents 11–18% and 10–20% of whole wheat and rye kernels, respectively (Brouns et al. 2012; Delcour and Hoseney 2010). Wheat grains can also be pre-treated by debranning using so-called modified rice polishers (as reviewed by Dexter and Wood 1996). In debranning, the grains are first conditioned for a short time, which then allows layer-by-layer separation of the outer grain layers, resulting in the by-products pericarp and aleurone. The relatively pure aleurone fraction from the process is enriched in protein (19.0%) compared with the first outmost layer removed (12.9%) (Rizzello et al. 2012).

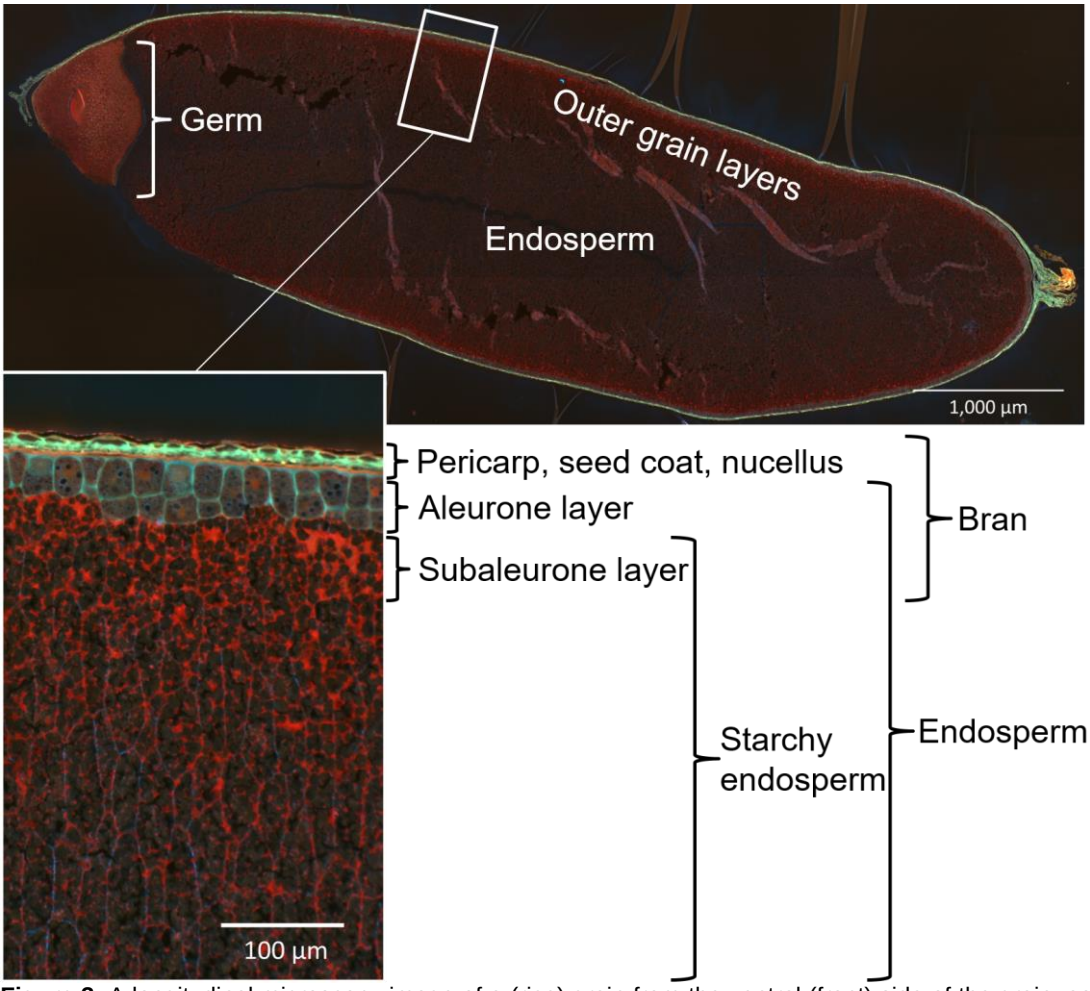

Figure 3. A longitudinal microscopy image of a (rice) grain from the ventral (front) side of the grain, as well as a closer image of the outer grain layers stained with Acid Fuchsin and Calcofluor White, showing proteins as red and cell wall glucans as blue, respectively. The pericarp layer appears as light green and yellowish, and the orange strand indicates the cutin layer. The starch is unstained and appears black (image courtesy of VTT Technical Research Centre of Finland, Dr. Ulla Holopainen-Mantila).

The separation of bran and germ from brown rice is carried out by pearling, after which the pearled grains can be further polished to remove remnants of the bran resulting in the white rice (i.e. the head rice). Rice bran and polish represent approximately 8 and 2% of paddy rice, respectively, the amounts of which vary depending on the applied milling procedures (Fujino 1978; Marshall and Wadsworth 1993; Saunders 1990). Dry milling of brown rice results in a combination of bran, germ and polish fractions, which are together called rice

bran. The main product from rice milling, white rice, is usually consumed directly as food. Prior to the dehulling or bran removal steps, the rice grain can be parboiled (i.e. soaked and boiled or steamed) to facilitate nutrient absorption from the outer grain layers into the endosperm, which fortifies the white rice with water-soluble vitamins (Juliano 1993).

As reviewed by Chinma et al. (2015), relatively large compositional variations are observed within each cereal bran (Table 2). The starch content of the brans differs due to the milling procedures applied for the bran separation, and wheat, rice and rye brans contain 9–25%, 10–20% and 13–40% starch, respectively (Kamal-Eldin et al. 2009; Nordlund et al. 2013b; Saunders 1990). Rice bran exhibits the highest oil and ash content of these three brans (Chinma et al. 2015; Juliano and Bechtel 1985). DF is the most abundant in wheat bran, and for all brans, DF is mainly composed of insoluble dietary fibre (IDF) (Abdul-Hamid and Luan 2000; Chinma et al. 2015). In wheat and rye brans, arabinoxylan is the most abundant DF class, representing 19–30% and 16–25% of the bran, respectively, and other bran cell wall constituents include cellulose, fructan, β-glucan and lignin (Bataillon et al. 1998; Kamal-Eldin et al. 2009; Nordlund et al. 2012). In rice bran, hemicelluloses (mainly arabinoxylan), cellulose and lignin are the most prominent cell wall components, constituting 40, 30 and 21% of the cell wall, respectively. In addition, pectin is present, and the β-glucan content is negligible (Shibuya et al. 1985). Bran proteins are discussed in more detail in Section 2.3.1.

Table 2. The biochemical composition of wheat, rice and rye brans.

	Wheat bran	Rice bran	Rye bran
Protein (% dm)	10–17[a]	12–16[a]	15[a]
Total dietary fibre (% dm)	48[a]	27[b]	36[a]
Soluble dietary fibre (% dm)	2[a]	2[b]	5[a]
Insoluble dietary fibre (% dm)	46[a]	25[b]	31[a]
Ash (% dm)	4–7[a]	7–10[a]	4[a]
Fat (% dm)	3–5[a]	17–23[c]	3[a]
Carbohydrates (% dm)	51–59[a]	31–52[a]	58[a]

dm: dry matter.
[a] reviewed by Chinma et al. (2015).
[b] according to Abdul-Hamid and Luan (2000).
[c] reviewed by Juliano and Bechtel (1985).

2.1.2 SIDE STREAMS FROM OTHER DRY SEPARATION PROCESSES OF CEREAL MATERIALS

The dry separation processes of cereal grains can target the enrichment of one or more main grain components, such as DF, protein or starch. In the simplest approaches, milling is combined with one or several sieving steps in order to enrich fibres in the largest particle-sized coarse fraction while protein or starch enrich in the small-sized fine fraction. In addition to sieving, the

separation of components may also be obtained via air classification (discussed in more detail in Section 2.2.2). Both of these techniques can also be applied for bran separation from whole grain (such as oat) flours (Girardet and Webster 2011). Another example is the enrichment of β-glucan from β-glucan-rich grains, barley and oats, reported by several authors and in various patented processes (Andersson et al. 2000; Kaukovirta-Norja et al. 2008; Knuckles and Chiu 1995; Lehtomäki and Myllymäki 2010; Mälkki et al. 2001; Nordlund et al. 2012; Sibakov et al. 2011; Vasanthan and Bhatty 1995; Wu et al. 1994; Wu and Doehlert 2002). Moreover, the processes may also aim at protein or starch enrichment.

DF enrichment from cereal grains by dry means produces side stream fractions that are high in other nutritionally valuable components, such as protein and starch. These fractionation processes often consist of multiple separation steps. In general, for barley and oat or oat bran fractionations, it can be stated that the first fine fractions in these processes are typically enriched in protein whereas DF and β-glucan enrichment occurs in the last steps, into coarse fractions (Vasanthan and Bhatty 1995; Wu et al. 1994; Wu and Doehlert 2002). Starch and DF fractionate first together, and later, when targeting the separation of DF from starch, starch is enriched in the fine fraction (Vasanthan and Bhatty 1995). Thus, considerable amounts of fractions containing components other than the target components are produced. Furthermore, in the fractionation processes of barley and oats, the DF content often decreases when starch content increases and vice versa (Wu et al. 1994; Wu and Doehlert 2002). Valorisation of these generated side streams by further enriching desirable components is regarded to be a promising approach to improve the food applicability of the side stream fraction. In the patented process described by Kaukovirta-Norja et al. (2008), non-heat-treated oats extracted by supercritical carbon dioxide (SC-CO_2) are fractionated in a two-step air classification or sieving process to obtain a fraction with 25–60% β-glucan content. The starch-enriched, starchy endosperm-derived fraction from the first fractionation step (i.e. the side stream from β-glucan extraction) can be further dry fractionated to obtain a fine fraction enriched in protein (up to 30–80%) and having an over 50% lower carbon footprint (kg/protein) when compared with dairy proteins (as analysed in a life cycle assessment-based study by Heusala et al. 2019).

2.1.3 OPPORTUNITIES AND CHALLENGES OF CEREAL SIDE STREAMS FOR FOOD USE

The side streams from dry cereal grain processing, including brans and other residual fractions, are valuable for food use for various reasons. Brans contain significant amounts of nutritionally relevant components, such as proteins, DF, vitamins and minerals (Chinma et al. 2015). In particular, the aleurone layers of cereal brans contain higher amounts of protein than the outer grain layers or starchy endosperm (Buri et al. 2004; Bushuk 2001; Juliano and Bechtel

1985). Moreover, the nutritional quality of wheat and rice aleurone proteins is regarded to be superior to that of the starchy endosperm proteins owing to the elevated amount of the essential and normally limiting amino acid in cereal grains, lysine, present in the aleurone (Buri et al. 2004; Han et al. 2015; Wang et al. 1999). As reviewed for wheat bran by Hemery et al. (2007), the outer grain layers, pericarp, seed coat and aleurone, are also enriched in DF, vitamins B and E, and minerals, as well as phenolic constituents and antioxidants.

The main drawbacks in the food applicability of cereal brans include their negative impact on the flavour and aftertaste of food products (Heiniö et al. 2016, 2003; Nordlund et al. 2013a) and their limited techno-functional properties (Rosa-Sibakov et al. 2015a). In addition to beneficial grain components, antinutritional factors are also enriched in the outer grain layers (Fardet 2010; Hemery et al. 2007). Phytic acid (*myo*-inositol hexakisphosphate), the main storage form of phosphorus in plants, is known to bind minerals and proteins due to their opposite charges, thus potentially having a negative impact on bioavailability (Kies et al. 2006; Selle et al. 2000). Phytic acid is mainly located in aleurone grains in rice, wheat and rye brans (Antoine et al. 2004b; Bohn et al. 2007; Parker 1981), which may reduce bran protein bioavailability. Other antinutritional factors located in bran include trypsin inhibitors, polyphenols, oxalates, saponins, lipases and haemagglutinin (Fardet 2010; Kaur et al. 2015). On the other hand, some of the beneficial bioactive bran components may be entrapped within the bran matrix and are thus not bioavailable (Fardet 2010). Outer grain layers may also include contaminations, such as microbes, mycotoxins, heavy metals and pesticides (Hemery et al. 2007; Laca et al. 2006). In regard to rice, the high lipid content, rendering the bran prone to rancidification due to presence of lipid-modifying enzymes in the native bran, restricts its food use (Malekian et al. 2000). Lipid oxidation is usually prevented by stabilising the full-fat bran using heat treatments, such as extrusion or parboiling. Another approach to avoid lipid oxidation is the removal of the bran oils by using, for example, solvent extraction. However, heat treatment, typically included in both stabilisation and extraction phases, negatively affects the bran quality by, for example, lowering protein solubility (Anderson and Guraya, 2001) and altering the amino acid profile (Gnanasambandam and Hettiarachchy, 1995). Other factors limiting the food use of rice bran are its potentially high microbial load and high content of silica due to hull contamination (Oliveira et al. 2012).

2.2 DRY FRACTIONATION OF CEREAL GRAINS

Dry processing to separate the components of plant materials has gained interest due to sustainability and energy-efficiency reasons, especially in regard to plant protein concentration. Dry fractionation technologies, including the unit operations of dry milling, air classification, sieving and electrostatic

separation, for protein enrichment from low-moisture plant materials consume considerably less energy compared with wet fractionation (i.e. aqueous extraction of proteins), as reviewed in Schutyser et al. (2015) and Schutyser and van der Goot (2011). The increased energy consumption in wet processing derives from the sum of the energy used in the milling, solid–liquid separation, precipitation and drying steps, of which drying is the most energy-intensive step. Moreover, the employment of harsh treatment conditions, such as high alkaline pH, high temperature and organic solvents, in wet extraction negatively affects both technological and nutritional quality of plant proteins (Deleu et al. 2019; Finley and Kohler 1979; Kornet et al. 2021; Wu and Inglett 1974). On the contrary, dry fractionation yields macromolecules that remain in native conditions, and also, retains most of the micronutrients during processing. Unit operations involved in dry fractionation processes are elucidated in the following Sections 2.2.1–2.2.4.

2.2.1 DRY MILLING FOR PARTICLE SIZE REDUCTION

Particle size reduction is a key unit operation in the dry processing of cereal raw materials. In addition to the term dry milling referring to the processes employed for refined flour production, it is also applied when performing particle size reduction for cereal raw materials by dry means. For that purpose, the milling is usually carried out using roller mills, hammer mills, impact mills (such as pin disc mills) or jet mills. The selection of mill is based on the targeted particle size distribution and following ingredient processing (namely, application in different food categories or as a raw material in dry fractionation). The level of particle size reduction also affects the properties of the further fractionated ingredients (see Sections 2.2.2–2.2.4).

Reduction of particle size has a prominent impact on the food applicability of cereal ingredients via modifications of the techno-functional, sensory and/or nutritional qualities. Effective milling may modify IDF into soluble dietary fibre (SDF), as has been reported in literature on wheat brans (Junejo et al. 2019; Van Craeyveld et al. 2009; Zhu et al. 2010) and rye brans (Alam et al. 2014). Similarly, impactful milling results in increased amounts of damaged starch in cereal flours (Berton et al. 2002; Drakos et al. 2017a; Niu et al. 2014; Tester 1997). This may in turn alter the functional and rheological properties of starch, for example, by increasing the water absorption of the flours (Berton et al. 2002; Drakos et al. 2017a; Niu et al. 2014), and by affecting the behaviour of the dough (Barrera et al. 2007) and starch susceptibility to hydrolysis by α-amylases during bread making (Barrera et al. 2016). Particle size reduction also has varying impacts on the techno-functional properties of proteins. The liberation of protein components from inside the insoluble and hard wheat bran cell wall structures by ball milling results in increased protein solubility (De Brier et al. 2015). Similarly, protein extractability from defatted rice bran at pH 11 increased from 59 to 69% as a result of roller milling (Prakash and Ramanatham 1994). On the contrary, too intensive jet milling has been linked

with the reduced solubility of rye flour proteins (Drakos et al. 2017b) as harsher grinding conditions are associated with heat generation modifying protein properties or resulting in aggregation of the polymers (Van Craeyveld et al. 2009). Particle size reduction also alters hydration properties, and oil binding capacity (OBC) and water binding capacity (WBC) of cereal ingredients. Wheat brans with a smaller particle size have been shown to exhibit lower WBC due to modification of the fibrous matric structure and the potential impact of particle size reduction on the properties of biomolecules (Auffret et al. 1994; De Bondt et al. 2020; Zhang and Moore 1997; Zhu et al. 2010) whereas, for some materials, the opposite may also apply as surface area increases (as reviewed by Elleuch et al. 2011). Similarly, lowered physical entrapment of oil, that is, lowered OBC, has been attributed to the reduced particle size of rye flour (Drakos et al. 2017a).

Particle size considerably affects the technological properties of cereal ingredients, especially brans. For example, their behaviour in baking and extrusion, as well as dispersion stability in high-moisture food systems are altered, as reviewed by Doblado-Maldonado et al. (2012) and Chinma et al. (2015), and the impact of particle size varies depending on the application studied. The modified food applicability of ingredients with different particle sizes results from the biochemical and physical modifications caused by the milling. Further, the mechanical liberation of cell constituents from the grain structures also affects the technological ingredient properties. The incorporation of wheat bran of various particle sizes into wheat bread has revealed ambiguous effects on bread-making quality and dough-mixing properties. In general, addition of bran impairs bread quality, but opposing impacts of the particle size on, for example, dough development time have been reported (Coda et al. 2014a; Jacobs et al. 2016). Regarding the impact of particle size on bread volume, findings suggesting both an optimal particle size (Coda et al. 2014a) and the negligible impact of the size (Curti et al. 2013) have been reported. In the study by Noort et al. (2010), the negative impact of finely milled bran on bread-making quality was postulated to potentially result from the increased surface area of the bran particles increasing the susceptibility to interactions and the release of reactive compounds affecting gluten network formation. In extrusion, the reduction of bran particle size increased the expansion, crispiness and porousness of puffed rye bran extrudates (Alam et al. 2014). In regard to high-moisture food systems, particle size has a prominent role in the solubilisation of the components and, further, in the stability of the systems. The improved colloidal stability of cereal ingredient dispersions has been reported to result from, for example, the microfludisation-aided particle size reduction of wheat bran (De Bondt et al. 2020; Rosa-Sibakov et al. 2015b). In addition, the particle size reduction of bran ingredients results in improved mouthfeel (Coda et al. 2014a).

Decreasing particle size may also improve the bioavailability of micro- and macronutrients, as reviewed by Capuano and Pellegrini (2019), Hemery et al. (2007) and Joye (2019). Junejo et al. (2019) reported wheat bran to be more

prone to α-amylase, pepsin and pancreatin enzymes after ultra-fine milling compared with non- or coarsely milled bran, and Latunde-Dada et al. (2014) showed 52% higher iron bioavailability from micro-milled wheat aleurone flour than from lesser milled wheat aleurone flour. Likewise, the antioxidant capacity of wheat bran was shown to increase as a result of particle size reduction due to the improved exposure of the phenolic residues responsible for the antioxidant properties (Rosa et al. 2013), and the use of finer bran in breads has improved the bioaccessibility of phenolic acids (Hemery et al. 2010). Coda et al. (2014b) observed that, for wheat bran, micronisation to the median particle size of 400 µm was optimal when considering the *in vitro* protein digestibility, while lower digestibilities were reached for brans with both the smaller and larger particle size medians of 750, 160 and 50 µm.

Milling for the disintegration of the cellular grain structures is a prerequisite for successful component separation in dry fractionation. Requirements for particle size reduction prior to subsequent dry fractionation are dependent on the raw material properties and desired component separation, and usually a different size reduction level is targeted when aiming at protein, fibre or histological component enrichment. Moreover, the selection of milling technique may affect the amount of damaged starch formed, result in mono- or bimodal particle size distribution and have a significant impact on the material flowability inside the fractionation equipment (Tenou et al. 1999). The different approaches for the dry fractionation of cereal materials are introduced in more detail in the following sub-sections (Sections 2.2.2–2.2.4).

2.2.2 AIR CLASSIFICATION FOR CEREAL COMPONENT FRACTIONATION

Air classification is a dry separation method based on the fractionation of heterogeneous particles from a solid disperse phase into two fractions, fine and coarse fractions, due to the settling velocities of the particles which are determined by their sizes, densities and shapes (Furchner and Zampini 2012; Schutyser and van der Goot 2011). For plant material fractionation, the most commonly utilised air classifiers are centrifugal classifiers (i.e. deflector-wheel air classifiers; Figure 4), in which the drag forces of the air cause the transfer of the fine particles through the classifier wheel until they reach a cyclone, while for coarse particles, the centrifugal forces predominate and restrict particle transfer through the wheel, resulting in their gravitation downwards. Adjustments to the targeted particle size range of the fractions are made by modifying the classifier wheel speed, which determines the material fractionation, whereas the air flow rate is usually kept constant and high unless an extremely fine particle size of the fine fraction is desired, as reviewed by Furchner and Zampini (2012). Another important aspect in air classification is the optimisation of the inlet material particle size, which determines the behaviour and fractionation of the material during air classification. In separation aiming at protein enrichment, the cut size (i.e. the particle size that

has a 50% probability to enter to both fine and coarse fractions) is generally 10 µm (Schutyser and van der Goot 2011). The performance of the air classification process is evaluated by determining mass yields and the component concentrations in the fractions, as well as component separation efficiencies, such as protein separation efficiency (PSE) and starch separation efficiency (SSE) (Tyler et al. 1981). The scalability of air classification is considered to be good due to the relatively high production capacity, reaching 500 t/h for the fine fraction at industrial scale, compared with 100 g/h at laboratory/pilot scale (Furchner and Zampini 2012).

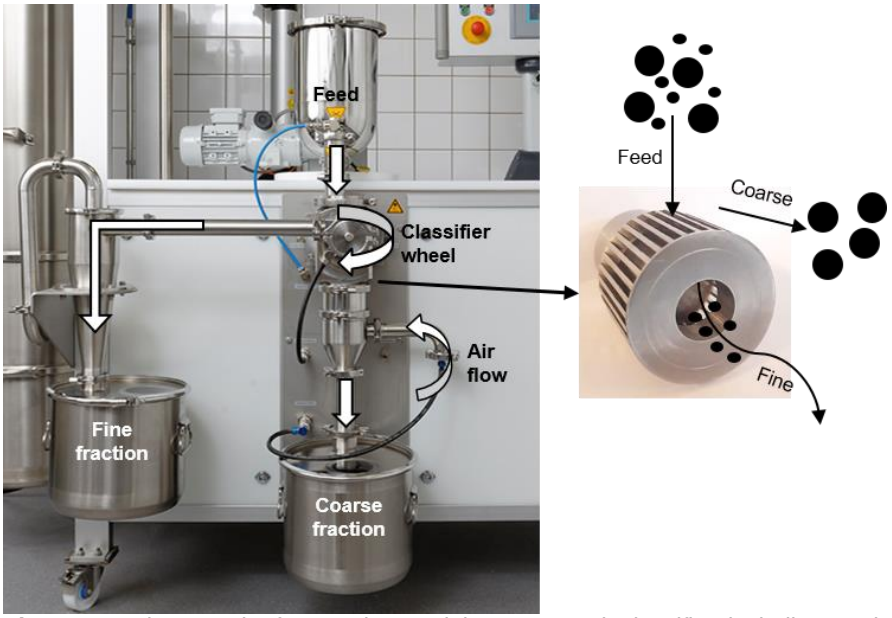

Figure 4. A photograph of a Hosokawa-Alpine 50 ATP air classifier, including a schematic presentation of the material flow and adjustable processing parameters of the equipment (image courtesy of VTT).

In food technology, air classification has been successfully utilised for protein, starch and fibre separation and enrichment. The targeted end-product properties (i.e. it being enriched in protein, starch or fibre) determine whether it is the fine or the coarse fraction that is considered the final product. However, from process feasibility and sustainability perspectives, it is important to consider the applicability of both fractions and determine suitable product and application opportunities for each fraction. In addition to component fractionation, air classification has also been employed for the histological separation of different grain components and, more specifically, bran layers. For example, Bohm et al. (2003) described a dry process for wheat bran that included tempering and several dry processing steps (milling, air classification, electrostatic separation) that allowed the production of a relatively pure aleurone ingredient that was free from other bran components.

Protein enrichment by air classification from plant materials has been investigated especially in the case of pulses in which the main individual sub-cellular components (starch granules and protein bodies) considerably differ in their sizes. The rather monomodal and large size distribution of starch

granules in pulses allows their better separation from small-sized protein bodies when compared with cereal grains in which the starch granules mostly exhibit bimodal size distributions (Schutyser and van der Goot 2011). In rice, free starch granules are particularly small (3–9 µm) whereas multiple granules may be present within one amyloplast, sizing 7–39 µm (Juliano 1985; Saio and Noguchi 1983). In wheat, rye and barley, starch is present as large A-type granules (up to 40, 48 and 50 µm, respectively) and smaller B-type granules (up to 10, 12 and 10 µm, respectively) (Goering et al. 1973; Heneen and Brismar 1987; Takeda et al. 1999), which usually results in their fractionation during air classification into coarse and fine fractions, respectively (Vasanthan and Bhatty 1995). Moreover, cereal endosperm structure further hinders protein fractionation due to the embedment of small starch granules within a matrix formed of storage proteins (Darlington et al. 2000). In the aleurone and subaleurone region of the endosperm, cereal proteins are located inside small-sized (0.5–5 µm) protein bodies (Juliano and Bechtel 1985; Pernollet 1978), the sizes of which partially overlap with small starch granules.

Despite the previously described factors hindering protein fractionation from cereal grains, various attempts at enriching protein have been reported. Research has mainly concentrated on the fractionation of endosperm or whole grain flours, whereas only a little research is available on bran protein enrichment. Table 3 shows multiple examples of the air classification of cereal grains for protein enrichment, revealing the protein contents, mass yields and protein separation efficiencies obtained in fractionation, and it also discriminates the processing steps applied prior to air classification. As summarised in Table 3, the highest protein content obtained from wheat, rice and barley varies between 16–40%, whereas significantly higher content of 73–83% is reported for oats (Sibakov et al. 2011; Wu and Stringfellow 1973). However, the distinctive feature of air classification related to the relationship between the mass yield and protein content of the protein-enriched fine fraction is observed, especially in the research related to oats, in which the high protein contents are obtained at the expense of the low mass yields and PSEs of the protein-enriched fraction (Sibakov et al. 2011; Wu and Stringfellow 1973). It is noteworthy that relatively little or no research is available concerning the air classification of other cereal grains, such as corn (Garcia et al. 1972) or sorghum (Stringfellow and Peplinski 1966).

Table 3. Examples of protein and starch enrichment from cereal grains by air classification, including information about the raw material, pre-treatments, number of air classifications performed and the contents, yields and component-separation efficiencies in the produced fractions.

Raw material	Content in raw material (% dm)	Pre-treatment	Fraction obtained during	Contents in the fine fraction (% dm)	Contents in the coarse fraction (% dm)	Mass yield of the fine fraction (%)	Mass yield of the coarse fraction (%)	PSE in the fine fraction (%), SSE in the coarse fraction (%)	Reference
Wheat	Protein: 10–18; starch: na	3 x 14 000 rpm pin disc milling	2nd step of a 5-step AC	Protein: 23–33	na	11–25	na	PSE: 22–45*	Wu & Stringfellow (1992)
Wheat bran	Protein: 14.9; starch: na	Grinding	Combination of fine fractions from 2-step process (1st coarse reprocessed)	Protein: 22.1	na	14	na	PSE: 20.8*	Ranhotra et al. (1994)
Wheat	Protein: 11.4–12.5; starch: na	Jet milling of raw material before AC and coarse fractions after AC	1-5 steps of ACs	Protein after 1st AC: 20.7–22.9	Protein after the 5th AC: 1.5–2.5	after the 5th AC: 3.5–4.4	after the 5th AC: 24.4–29.5	Total PSE to the coarse fraction after the 5th AC: 3.9–4.9*	Létang et al. (2002)
Wheat	Protein: 12.9; starch: na	Commercial wheat flour	1-step AC	Protein: 17.5	Protein: 12.7	10	90	PSE: 13.6*	Lundgren (2011)
Rice flour	Protein: 14; starch: na	Grain polished to 75%	1-step AC	Protein: 17.5	na	na	na	na	Noguchi et al. (1981)
Flour from polished rice	Protein: 7.4; starch: na	Frozen milling by impact mill followed by 2 x jet milling	1-step AC	Protein: 9.7	Protein: 4.7	45	55	PSE: 59.0*	Saio & Noguchi (1983)
Rice bran	Protein: 14.7; starch: na	Sonication-aided hexane defatting and impact milling	1-step AC of <73 µm material or jet milling and one-step AC of <73 µm material	Protein: 15.6–16.2	Protein: 13.8–14.5	na	na	na	Saio & Noguchi (1983)
Rice flour	Protein: na; starch: na	Fluidised bed opposed jet milling	1-step AC	Protein: 19.8	Protein: 10.0	na	na	na	Park et al. (1993)
Barley, dehulled	Protein: 14.8; starch: 68	Pin disc milling	1-step AC	Protein: 40.1; starch: 34	Protein: 10.2; starch: 72	17.5	82.5	PSE: 47.4*, SSE: 87.4*	Vose & Youngs (1978)

Malted barley, dehulled	Protein: 11.7; starch: 60	Pin disc milling	1-step AC	Protein: 26.8; starch: 38	Protein: 9.5; starch: 64	15.6	84.4	PSE: 35.7*; SSE: 90.0*	Vose & Youngs (1978)
Hulless barley	Protein: 22.4; starch: 24.8	Defatting, pin disc milling	5-step AC	Protein: 26.8 in the 1st fine fraction; starch: 42.5	na	22.0	na	PSE: 26.3*	Wu et al. (1994)
Dehulled barley	Protein: 10.9; starch: 66.2	Pin disc milling	5-step AC	Protein: 23.1–28.4 in the 1st fine fraction; starch: 57.3–60.5	na	4.7–8.0	na	PSE: 15.4*	Wu et al. (1994)
Hulless barley	Protein: 18.6; starch: 54.5	Pin disc milling	5-step AC	Protein: 28.5 in the 1st fine fraction; starch: 52.6	na	9.4	na	PSE: 14.4*	Wu et al. (1994)
Barley	Protein: 12.3–13.6; starch: 53.6–62.0	Pin disc milling	3-step AC	Protein: 17.5–27.7 in the 1st fine fraction	Starch: 59.1–70.5 in the 1st coarse fraction	6.8–22.8	75.1–87.0	PSE: 15.0–32.4*; SSE: 83.9–98.9*	Vasanthan & Bhatty (1995)
Barley	Protein: 9.0–17.6; starch: 31.0–63.7	0.7 mm sieve milling and impact milling	5-step AC	Protein: 14.0–20.6 in the 1st fine fraction	Starch enriched in the 3rd fine fraction, content: 70–80%	15.3–36.1	na	PSE: 15.1–51.2*	A. A M Andersson et al. (2000)
Dehulled oat grain (i.e. oat groat)	Protein: 16.3–22.7; starch: na	Pentane-hexane defatting, pin disc milling	1st step of AC	Protein: 83.1–83.3 in the filter fraction, 20.8–29.4 in the fine fraction	na	filter fraction: 2; fine fraction: 26	na	PSE: filter fraction 7.3–10.2; fine fraction 33.2–33.7*	Wu & Stringfellow (1973)
Non-heat-treated, dehulled oat	Protein 17.2; starch: 65.6	SC-CO_2 extraction, pin disc milling	2-step AC	2nd step fine fraction, protein: 73.0; starch: 17.1	2nd step coarse fraction, protein: 10.7; starch: 77.2	5.0	76.0	PSE: 21.2*; SSE: 89.4*	Sibakov et al. (2011)

dm: dry matter; PSE: protein separation efficiency; SSE: starch separation efficiency; na: not analysed; * calculated in this work based on the data provided in the article; AC: air classification.

The fractionation of DF by air classification, especially from cereals rich in β-glucan, such as oats and barley, has been extensively studied and Table 4 collects examples on those fractionations found in literature. The enrichment of DF takes place in the coarse fraction in air classification and multiple rounds of subsequent air classifications, which commonly include milling steps in between the air classifications, facilitate the separation. This is seen as an increase in the obtained DF content, whereas the mass yield decreases. Indeed, the reported air classification processes have mainly relied on multiple separation steps and have allowed β-glucan enrichment up to 24, 31, 34 and 40% from dehulled barley, hulless and defatted barley, defatted oat groats and defatted oat bran, respectively (Sibakov et al. 2014, 2011; Vasanthan and Bhatty 1995; Wu et al. 1994). In addition, successful enrichment and recovery of DF has been obtained from rye bran and kernels (Nordlund et al. 2013b, 2013a).

Studies targeting understanding the simultaneous behaviour of protein and DF in air classification remain scarce. Regarding the concurrent fractionation of other grain components, the enrichment of lipids (Chung et al. 2002; Létang et al. 2002; Ranhotra et al. 1994), damaged starch (Létang et al. 2002), and vitamins and phytic acid (Ranhotra et al. 1994) in the protein-enriched wheat flour fractions has been reported. Additionally, an increase in the ratio between SDF and IDF has been detected in the protein-enriched fraction (Létang et al. 2002; Ranhotra et al. 1992). Furthermore, the contents of all essential and semi-essential amino acids were shown to be higher in the protein fraction than in the original wheat bran, and for example, the amount of lysine was 44% higher and the protein efficiency ratio of 1.8 was close to that of casein (2.5) and exceeded the values reported earlier for wheat flour and bran (1.0–1.5) (Ranhotra et al. 1994).

Table 4. Examples of dietary fibre enrichment from cereal grains by air classification, including information about the raw material, pre-treatments, number of air classifications performed, and contents, yields and component separation efficiencies in the produced fractions.

Raw material	β-glucan content in raw material (% dm)	Pre-treatment	Amount of processing steps	β-glucan content in the fine fraction (% dm)	β-glucan content in the coarse fraction (% dm)	Mass yield of the fine fraction (%)	Mass yield of the coarse fraction (%)	β-glucan separation efficiency (%) into the coarse fraction	Reference
Hulless barley	19.6	Defatting, pin disc milling	5-step AC	37.9 in the 4th fine fraction	31.3 in the 5th coarse fraction	5.0	31.0	49.5*	Wu et al. (1994)
Dehulled barley	5.8	Pin disc milling	5-step AC	9.1 in the 4th fine fraction	14.7 in the 5th coarse fraction	19.3	13.2	33.5*	Wu et al. (1994)
Hulless barley	8.0	Pin disc milling	5-step AC	8.1 in the 4th fine fraction	14.6 in the 5th coarse fraction	14.3	27.3	49.8*	Wu et al. (1994)
Hulless or dehulled barleys	5.9–7.8	Pin disc milling	3-step AC	6.5–8.1 in the 3rd fine fraction	13.1–23.8 in the 3rd coarse fraction	43.7–63.4	7.6–20.9	23.1–58.7*	Vasanthan & Bhatty (1995)
Covered and naked barleys	3.8–17.0	0.7 mm sieve milling and impact milling	5-step AC	8.0–23.0 in the 5th fine fraction	4.5–23.5 in the 5th coarse fraction	8.0–33.4	2.0–9.4	Fine fraction: 16.8–45.2*; coarse fraction: 3.5–11.9*	Andersson et al. (2000)
Hulless barley	5.4–7.8 (TDF: 11.5–12.8)	Impact milling	1- and 2-step AC	na	1st step coarse fraction: 8.1–11.1 (TDF: 23.4–25.5); coarse fraction from the re-fractionation of the 1st step fine fraction: 11.2–15.6 (TDF: 21.8–25.5)	1st: 79.6–83.5; 2nd: 53.7–61.2	1st: 10.4–16.5; 2nd: 28.4–29.8	1st step coarse fraction: 14.8–24.8 (TDF: 20.7–33.6)*; coarse fraction from the re-fractionation of the 1st step fine fraction: 56.8–61.8 (TDF: 56.5–56.6)*	Ferrari et al. (2009)

Dehulled and pearled waxy barley	9.0	Pin disc milling	1-step AC	5.2	15.3	70	30	51.0*	Messia et al. (2020)
Oat bran (dehulled, steamed and hexane-extracted oats)	12.6	Pin disc milling	4-step AC	4^{th} fine fraction: 10.8	4^{th} coarse fraction: 18.8	4^{th} fine fraction: 4.7	4^{th} coarse fraction: 39.3	58.6*	Wu & Doehlert (2002)
Non-heat-treated dehulled oat	3.2	SC-CO_2 extraction, pin disc milling	2-step AC	1^{st} step: 1.3; 2^{nd} step: 11.4	1^{st} step: 21.3; 2^{nd} step: 33.9	1^{st} step: 81.0; 2^{nd} step: 6.5	1^{st} step: 14.3; 2^{nd} step: 7.8	1^{st} step: 95.2*; 2^{nd} step: 82.6*	Sibakov et al. (2011)
Non-heat-treated SC-CO_2-defatted dehulled oat	35.0	Ultra-fine milling	1-step AC	11.1	40.3	15.4	84.6	97.4*	Sibakov et al. (2014)
Oat bran	7.6 (total DF: 14.9)	Pin disc milling	3-step AC and air jet sieving	na	15.1 (total DF: 28.9)	na	na	na	Nordlund et al. (2012)
SC-CO_2-defatted oat bran	14.3	Pin disc milling	5-step AC	4.3–14.7 in the the 4 fine fractions	22.4	na	na	na	Stevenson et al. (2008)
Rye kernels	SDF: 4.7; IDF: 11.7	0.3 mm sieve milling	2-step AC	SDF: 4.9; IDF: 9.4	SDF: 7.9; IDF: 27.6	43	29	SDF: 48.7; IDF: 68.4	Nordlund et al. (2013a)

dm: dry matter; * calculated in this work based on the data provided in the article; AC: air classification; TDF: total dietary fibre; SC-CO_2: supercritical carbon dioxide; na: not analysed; SDF: soluble dietary fibre; IDF: insoluble dietary fibre.

2.2.3 COMPARISON OF DRY SEPARATION PROCESSES FOR FRACTIONATION OF COMPONENTS FROM CEREAL GRAINS

In addition to air classification, dry separation of cereal grain components can be achieved by sieving or applying electrostatic separation. In this section, these two methods are shortly presented, and their advantages and disadvantages when compared with air classification are evaluated in Table 5.

Sieving is a traditional dry fractionation method, employed for the separation of particles based on their sizes between one or several meshes of decreasing size. Sieving is considered the method with the most potential for dry particle separation when the particles exhibit sizes larger than 100–200 µm. Rhodes (2008) reviewed that normal or air-jet sieve sizes larger than 45 and 20 µm, respectively, provide reliable results in sieving-based particle size analysis. As reviewed by Furchner and Zampini (2012), sieving is technologically easier to carry out than air classification and requires less energy. However, the main limitations in sieving include clogging or blinding of the sieves, especially with high-fat and ultra-fine materials, and the separation mechanism being limited to only separating according to the size and shape differences of the particles. As proteins are found in the finest-sized particles and fibrous plant cell wall materials usually remain in larger cellular integrities, size-based separation and the enrichment of protein and fibre in the fine and coarse fractions, respectively, may be achieved via sieving (Schutyser and van der Goot 2011).

The fractionation of components in an electrostatic separator takes place as a result of differing electrical charges that the components receive during a charging step. The charges obtained are component and species specific and are mainly affected by the surface properties of the materials, such as surface chemistry, electrical conductivity and dielectric properties (Flynn et al. 2019; Mazumder et al. 2006; Németh et al. 2003). Plant material fractionation by electrostatic separation is based on either conductive induction, ion or corona charging, or tribo-charging. As reviewed by Barakat and Mayer-Laigle (2017), in a parallel-plate tribo-electrostatic separator particles are tribo-charged as a result of collisions of the particles with both each other and the equipment walls inside the charging pipe, and fractionation takes place in a separation chamber where the positively and negatively charged particles adhere to the high voltage electrodes of opposite charges and uncharged particles separate by gravity. In conductive belt or drum separators, particles with different conductivities are separated from each other (Flynn et al. 2019; Higashiyama and Asano 1998).

Table 5. Comparison of dry fractionation approaches.

Property	Air classification	Sieving	Electrostatic separation	References
Separation principle	Size, density and shape of particles	Size and shape of particles	Electrical charging properties of particles	Furchner & Zampini (2012)
Optimal raw material particle size	1–200 µm	Preferably larger than 100–200 µm; normal dry sieve sizes >45 µm and air jet sieve sizes >20 µm are applicable for particle separation	Smaller particles obtain higher charges, corona-belt separators are only applicable for large particles	Furchner & Zampini (2012), Hemery et al. (2009), J. Wang et al. (2014; 2015a), Rhodes (2008)
Energy requirements	0.01–1 MJ/kg during milling to median particle sizes 100–1 µm, 3–44 kJ/kg for air classification (of pulse protein)	Requires the least energy and is technologically the easiest to be carried out	No information available	Furchner & Zampini (2012), Schutyser & van der Goot (2011), Wang & Forssberg (2007)
Protein separation	More applicable to pulses than cereal grains. Separation of individual protein bodies (1–5 µm) is possible.	Limited due to the minimum particle size reachable (~20 µm)	More suitable for oil-rich pulses or oilseed press cakes, moderate enrichment from cereal grains and starch-rich pulses	Pelgrom et al. (2013, 2015), Assatory et al. (2019), Laguna et al. (2018)
Dietary fibre separation	Suitable for cereal grains, especially brans	Suitable for cereal grains, especially brans	Generally not much more efficient than sieving	Wu et al. (1994), Wang et al. (2016)
Industrial scalability	Industrial scale production exists, 90 000 t/a	Applicable in industrial-scale mills	Commercially significant processing rates not yet reached	Flynn et al. (2019), Schutyser et al. (2015)
Factors affecting separation	Similar sizes and densities of the particles that are to be separated (i.e. small starch granules and protein bodies) and high fat content of the material hinder separation	Clogging of the sieves (high-fat, ultra-fine), material amount, time, horizontal and vertical sieve movements, sieve amounts, moisture content, particle size, pre-milling steps and the composition of the raw material	Similar charges restrict separation, charging properties are species specific, the agglomeration of oppositely charged components and lipid liberation hinders separation, humidity and water content affect charging	Pelgrom et al. (2015), Wang et al. (2016), J. Wang et al. (2015a), Nomura et al. (2003)

Both the electrostatic separation and sieving studies reported in literature for cereal grains have mostly concentrated on DF enrichment. DF enrichment by electrostatic separation has been achieved for defatted and pin disc-milled rice bran (from 32 to 52% DF with a mass yield of 44% in a one-step process and from 32 to 67% DF with a mass yield of 21% in a two-step process) (Wang et al. 2016) and pin disc-milled wheat bran (from 23 to 31% DF with 16% mass yield) (J. Wang et al. 2015b), whereas the same authors revealed more successful separation by using only a simplier air jet sieving (68% DF with 22% mass yield for rice bran and 31% DF with 45% mass yield for wheat bran). Protein content was not considerably changed in any of the fractions (J. Wang et al. 2015b; Wang et al. 2016). For wheat bran, further improvements were obtained by adding an air jet sieving step after the electrostatic separation, which resulted in arabinoxylan content of 43% with an 8% mass yield (J. Wang et al. 2015b). Similar data supporting better DF enrichment by sieving than by air classification has also been reported for barley (Knuckles and Chiu 1995). On the contrary, Wu et al. (1994) observed for non-defatted and defatted high-β-glucan barley variety samples β-glucan enrichment from 17.4–19.6 to 24.6–28.3% in a sieving fraction with approximately 5% mass yield, whereas higher β-glucan contents and mass yields were obtained by air classification. In regard to oats, the sieving of a defatted and pin disc-milled oat bran allowed the production of several fractions with β-glucan content of 19.9–20.7% compared with 11.1% in the raw material (Wu and Doehlert 2002). Higher contents were reached in electrostatic separation by Sibakov et al. (2014) who demonstrated that defatting enhanced β-glucan separation from ultra-finely milled oat bran and the contents were increased from the original 21.3–35.0 to 26.3–42.2% and 31.2–48.4% with mass yields of 53.1–46.2% and 27.3–23.1% by applying one- and two-step electrostatic separation processes, respectively. On the contrary, air classification of the same materials allowed enrichment from 21.3 to 35.0% and, further, from 35.0 to 40.3% with mass yields of 54.5% and 84.6%, suggesting that from a high β-glucan material (35.0% β-glucan), more enrichment is achieved by electrostatic separation (up to 48.4%) than by air classification (up to 40.3%), whereas separation efficiencies were higher in air classification due to higher mass yields. On the contrary, in another approach that aimed at histological aleurone enrichment, several steps of electrostatic separations performed for impact-milled rye and wheat brans decreased the total DF content but increased the ratio of SDF and IDF (Nordlund et al. 2012).

Research on successful cereal protein enrichment by electrostatic separation is lacking in literature. For starch-rich pulses, air classification has proven more efficient than electrostatic separation, whereas electrostatic separation is more applicable for oil-rich pulses and oilseeds, such as rapeseed and sunflower seed press cakes (Laguna et al. 2018; Pelgrom et al. 2015). In regard to sieving, Prakash and Ramanatham (1994) were able to reach protein content of 18–20% in sieving fractions produced from a defatted and roller-milled rice bran that initially had 17% protein content, and Jayadeep

et al. (2009) produced a protein-enriched fraction (max. 19.3% protein, <5% mass yield) from a defatted and crushed rice bran (14.5% protein). Sumner et al. (1985) separated different pearling fractions from barley and reached a maximum protein content of 27.0% with 17% mass yield from the hulless non-waxy barley (19.9% protein). Sieving a high-protein, high-beta-glucan barley variety (23.3% protein) resulted in a fraction <64 µm with 26.6% protein with 44% mass yield, and protein enrichment was further improved by defatting the relatively high-fat (5.7% fat) barley material, that allowed to reach protein content of 29.1% with 15% mass yield in particles <43 µm, whereas higher PSE was obtained by air classification (Wu et al. 1994). Protein enrichment to 21.8% in an aleurone fraction produced from wheat bran (15.2% protein; Hemery et al. 2009) has been reached using different dry processes based on descriptions in Bohm et al. (2003) and Bohm and Kratzer (2005), and for rye, the fractionation of rye aleurone from whole rye flour by sieving resulted in protein enrichment from 11.4 to 17.6% (Glitsø and Bach Knudsen 1999). For defatted and pin disc-milled oat bran, the protein content has been increased from 28.6% to over 32% in multiple sieving fractions with low mass yields (Wu and Doehlert 2002).

2.2.4 FACTORS AFFECTING THE EFFICACY OF DRY FRACTIONATION

Challenges in dry processing are associated with the low component purity and mass yield of the fractions obtained. The behaviour of the raw material in fractionation can, however, be somewhat altered using different physical pre-processes or by exploiting the knowledge of the cellular architecture of the raw materials. In this section, special attention is paid to the impact of the fat removal, raw material selection, flow aid addition and moisture content on efficacy of dry fractionation.

High fat content of the raw material may hinder dry processing due to lowered particle dispersability (Dijkink et al. 2007; Schutyser and van der Goot 2011) or material adhesion to milling or fractionation equipment (Sibakov et al. 2011). For example, as stipulated in the patent of Flynn et al. (2019), the fat content of the raw material should not exceed 20% in electrostatic separation. Indeed, Sibakov et al. (2011, 2014) reported improved β-glucan fractionation from defatted oat groats and oat bran in both air classification- and electrostatic separation-based studies (Sibakov et al. 2011, 2014). Moreover, defatting intensified the extent of particle size reduction in pin disc milling (Sibakov et al. 2011). Likewise, higher β-glucan content has been obtained in the sieving of a defatted, high-fat barley variety (Wu et al. 1994).

The initial protein content of the starting material does not necessarily positively correlate with the maximum protein content obtained during air classification. For example, hard wheat varieties usually exhibit higher protein content than soft wheat varieties, whereas the protein fractions from soft wheat varieties reach higher protein separation efficiencies (Wu and Stringfellow

1992). A similar phenomenon has also been observed for various pulse materials (Tyler et al. 1981), whereas for wheats, the distinctive differences in the starchy endosperm morphology between soft and hard varieties affect the component separation in air classification considerably. In hard wheats, milling fractures the cellular structures first between the cell walls and, during further/more impactful milling, the starch granules begin to break and damage. In further fractionation, the broken granules separate together with protein in size-based dry processes. On the contrary, in the soft wheats, the fracture occurs in the middle of the cells, affecting and separating protein–starch interactions in the endosperm more efficiently (Piot et al. 2000). Létang et al. (2002) verified that the more pronounced separation of protein and starch took place with soft, rather than hard, wheat flour. Higher milling resistance was observed for hard wheat, and also, a more compact protein matrix, embedding the starch granules, was found for the hard wheat when compared with soft wheat, which showed more distinguishably separated protein and starch granules (Létang et al. 2002). Furthermore, puroindolines and hordoindolines, the specific proteins that interact with lipids on starch surfaces in wheat and barley endosperm, respectively, may affect the behaviour of the flour in dry processing. In soft grains they bind to starch, whereas in the hard grains they attach to the protein matrix, which increases interactions between protein and starch, and may further limit protein fractionation from hard grains (Darlington et al. 2000).

In dry fractionation the powder flowability is a critical physical property that influences the behaviour of the raw material during processing and handling (Dijkink et al. 2007). The size and density of the particles, both of which also play an essential role in dry fractionation, affect the flow characteristics of flours. Moreover, the water content of the powder material impacts on the flowability, and increased relative humidity decreases powder flowability (Dijkink et al. 2007). Particles with sizes lower than 200 µm, such as the flours utilised generally as a raw material in air classification, are considered to have cohesive properties that impair flowability (Rhodes 2008; Teunou et al. 1999). Flow aid components, such as fumed silica particles, adhering to the surfaces of larger particles and reducing the interparticle cohesion and van der Waals forces, have been proposed to improve the flowability of plant materials (Müller et al. 2008; Pelgrom et al. 2015).

The moisture content of cereal grains affects their physical properties, and the brittleness of materials is associated with lowered moisture content, which in turn requires less energy in grinding (Walde et al. 2002). Moreover, the improved susceptibility to particle size reduction as a result of drying prior to milling allows the production of higher-mass-yield, protein-rich fractions in the air classification of pulses, with only minor lowering impact on the protein content (Pelgrom et al. 2015; Tyler and Panchuk 1982). However, the optimum particle size for each separation process needs to be carefully considered, taking into account the impact of particle size reduction on, for example, the formation of damaged starch, which in turn lowers the enrichment of proteins

(Drakos et al. 2017a; Pelgrom et al. 2013; Wu and Stringfellow 1992). Additionally, the selection of milling style can have a remarkable impact on component separation during subsequent dry fractionation. For example, a comparison of grinding under cryogenic and ambient conditions revealed that, even though grinding under cryogenic conditions allows the easier particle size reduction of wheat bran, it only results in size reduction, whereas the bran layers (pericarp, seed coat, aleurone) stay adherent and are thus not able to be separated in the following fractionation steps (Hemery et al. 2011).

2.3 TECHNO-FUNCTIONAL PROPERTIES OF CEREAL INGREDIENTS

Traditionally, the technological functionality of cereal ingredients has been assessed from pure components and isolates, whereas several compounds present in the hybrid ingredients may contribute to the detected functionality. In the following Sections 2.3.1–2.3.3, the key functionalities associated with the main cereal components, especially proteins, but also starch and DF, are reviewed.

2.3.1 PROTEIN COMPOSITION AND SOLUBILITY

Plant proteins are generally classified as albumins, globulins, prolamins and glutelins, according to their solubilities in water, saline, aqueous ethanol and acid/base solvents, respectively, using a method originally described by Osborne (1924). The protein properties and quality differ between different cereal grains and also between the botanical and structural parts of the grain within each species. From the point of view of the plant, the proteins in the cereal grains act as storage, structural or metabolic/enzyme proteins. Bran proteins are typically considered to have a higher value than the endosperm proteins in relation to nutrition and technological functionality. Wheat bran (De Brier et al. 2015), rice bran (Amagliani et al. 2017), barley bran (Yupsanis et al. 1990) and rye bran (Bushuk 2001) contain higher amounts of water-soluble albumins and salt-soluble globulins, when compared with endosperm proteins with the same plant origin (Table 6). The albumins and globulins are generally richer in essential amino acids compared with prolamins and glutelins, which are most abundant in starchy endosperm fractions. For example, rice bran proteins show a higher protein efficiency ratio and contain higher amounts of certain essential amino acids than the proteins of rice endosperm (Han et al. 2015). Moreover, lysine, the limiting amino acid in cereal grains, exhibits 37% higher values in rice bran than in the starchy endosperm of rice (Han et al. 2015). Also for wheat, the bran proteins contain three times more lysine than the starchy endosperm proteins (De Brier et al. 2015; Rombouts et al. 2009). However, the bran proteins are known to have limited bioavailability due to their location inside the rigid cell wall structures, but bioprocessing, such as

enzymatic treatments and fermentation, have successfully been proven to improve the *in vitro* protein digestibility of bran proteins (Nordlund et al. 2013b).

Table 6. The distribution of cereal bran and starchy endosperm proteins within the Osborne protein solubility classes.

	Bran (% of protein)				Starchy endosperm (% of protein)			
	Alb.	**Glob.**	**Prol.**	**Glut.**	**Alb.**	**Glob.**	**Prol.**	**Glut.**
Wheat	24 (33)[a]	16 (33)[a]	11–18[a]	7–26[a]	11–16[e]	11–16[e]	50–53[e]	26–27[e]
Rice	24–42[b]	13–36[b]	1–8[b]	10–42[b]	4–6[f]	6–13[f]	2–7[f]	79–83[f]
Barley	(41–64)[c]	(41–64)[c]	8–18[c]	na	(16–28)[g]	(16–28)[g]	30–45[g]	14–31[g]
Rye	18[d]	30[d]	na	na	(26–30)[e]	(26–30)[e]	35–49[e]	8[e]

Alb.: albumins; Glob.: globulins; Prol.: prolamins; Glut.: glutelins.
[a] De Brier et al. (2015), Idris et al. (2003); [b] Adebiyi et al. (2009), Hamada (1997), C. Wang et al. (2014), Cagampang et al. (1966), Cao et al. (2009); [c] calculated based on pearling data in Holopainen et al. (2014) using content of 30% as the amount of glutelins (according to Shewry et al. (1988)) in the whole grain; na: not available; [d] Rohrlich and Rasmus (1956); [e] Lexhaller et al. (2019), Schalk et al. (2017); [f] Cagampang et al. (1966), Cao et al. (2009), Ju et al. (2001); [g] Celus et al. (2006) (whole grain flour), Lexhaller et al. (2019) (endosperm flour, sum of alb. and glob.: 24%), Linko et al. (1989) (whole grain flour), Schalk et al. (2017) (endosperm flour); all materials values within parentheses represent the sum of albumins and globulins.

Proteins possess a key role in defining the technological properties of a food ingredient as they affect the structure- and texture-forming abilities but also have an effect on the behaviour of the ingredient during the processing and manufacturing of foods, as well as on the final product stability. Protein solubility in water is often considered a pre-requisite for other techno-functional protein properties, such as gelling, emulsifying and foaming. Unfortunately, the solubility of plant proteins is usually much lower than that of proteins of animal origin. As reviewed by Day (2013) and Zayas (1997), protein solubility is determined by both intrinsic protein properties, such as amino acid composition, conformation, molecular weight and surface properties affected by the amounts of polar and non-polar amino acid groups, and external factors, such as pH, ionic strength, the solvent and temperature. At the isoelectric point, the net surface charge of the protein molecule is zero and protein is non-soluble. At pH values below or above the isoelectric point, the increase in net positive and negative charges, due to the protonation of carboxyl groups and deprotonation of amino groups, respectively, render the proteins soluble as a result of electrostatic repulsion and increased interactions with water (Zayas 1997). The solubility curves of plant proteins plotted as a function of pH are typically U-shaped even though higher solubilities are often observed at highly alkaline, rather than highly acidic, pH. For plant proteins, the isoelectric points are generally close to the food-relevant pH values, such as pH 4–8 for rice bran (Adebiyi et al. 2007; Chandi and Sogi 2007; Ju et al. 2001), pH 4–5.5 for wheat bran (Arte et al. 2019; Idris et al. 2003) and pH 5–6 for barley proteins (Bilgi and Çelik 2004; Wang et al. 2010; Yalçin et al. 2008), which limits direct plant protein ingredient applicability in the neutral and slightly acidic pH region.

2.3.2 TECHNOLOGICAL FUNCTIONALITY OF PROTEINS

Protein solubility usually correlates well with other techno-functional protein properties, such as gelation, emulsification and foaming, and those functionalities are also affected by both intrinsic and extrinsic factors. In gelation, the intermolecular covalent or non-covalent interactions (hydrophobic and electrostatic interactions, and hydrogen bonding) between the proteins in a colloidal dispersion increase through acidification, heating or salt addition, which leads to the formation of a network entrapping water (Foegeding and Davis 2011). During heat-induced gelation with a sufficient protein concentration, the proteins are first denatured or partially unfolded by heat, resulting in exposure of the hydrophobic parts of the protein, after which the molecules associate and aggregate, mainly due to hydrophobic and other physical and chemical interactions (reviewed by Totosaus et al. 2002). The pH and ionic strength of the protein dispersion have a considerable impact on the gel strength and structure. At low ionic strength and pH far from the isoelectric point, the repulsive forces dominate, which prevents the formation of large aggregates and allows the formation of a fine-stranded gel network, whereas particulate gels are formed close to the isoelectric point and at high ionic strength, as has been shown for pea, canola and quinoa proteins (Mäkinen et al. 2016; Munialo et al. 2015; Yang et al. 2014). Acid- and salt-induced gelations are often preceeded by a heating step at a protein concentration that does not allow heat-induced gelation but rather leads to partial unfolding, the exposure of hydrophobic residues and the formation of small soluble aggregates (Hickisch et al. 2016; Liu et al. 2004; Schmitt et al. 2019). After that, the addition of salts or acidification (by, e.g. fermentation or the addition of acidulants) result in increased attractive forces between the proteins due to shielding of the charges or reduced charges, respectively (Schmitt et al. 2019; Totosaus et al. 2002). The gelation of proteins is usually assessed by analysing the least gelation concentration (Sun et al. 2017; Yeom et al. 2010) or by small deformation oscillatory measurements that yield information about the gel viscoelastic properties via a storage modulus (G') and a loss modulus (G"), as well as large deformation measurements that provide insights into the gel strength (Rao 2014). Moreover, the water retention properties of the gel may be determined by observing spontaneous syneresis or analysing the water holding capacity (WHC), which provides information about the water retention capacity of the gel against a centrifugal force (Ercili-Cura et al. 2013).

Protein-stabilised emulsions and foams are formed due to the adsorption of surface active proteins onto oil–water or air–water interfaces, respectively. As reviewed by Damodaran (2005), Foegeding and Davis (2011) and Kinsella (1976), in emulsification and foaming, the proteins adsorb and orientate themselves to the interface, exposing the hydrophobic and hydrophilic moieties to the oil/gas and aqueous phases, respectively. Generally, high protein solubility aids in emulsification and foaming (Foegeding and Davis 2011). Analysis of emulsion capacity, describing the effectiveness of the emulsification process, is carried out by defining the maximum oil amount that

can be incorporated in an aqueous ingredient solution or by turbidity-based analysis of dilute emulsions (McClements 2007; Pearce and Kinsella 1978). Emulsion stability can be assessed by, for example, analysing particle size or sedimentation/creaming visually or using light scattering-based optical profiling (McClements 2007). In foaming, the capacity describes the maximum foam height obtained after whipping/stirring the aqueous ingredient dispersion, whereas stability is assessed by evaluating the decrease of foam height or the drainage (Arte et al. 2019; Nisov et al. 2020; Wang et al. 2010; C. Wang et al. 2014). Regarding the efficiency of emulsification and foaming as a function of pH, various studies have reported similar U-shaped curves to those that are commonly observed for the protein solubility of cereal proteins (Bilgi and Çelik 2004; Idris et al. 2003; Wang et al. 2010; Yeom et al. 2010).

The WBC and OBC, assessed by centrifugation-based methods, may be analysed from pure protein ingredients in order to predict the behaviour of the protein in foods, but in multicomponent ingredients, other components also considerably affect these properties. The WBC, which is an important property in, for example, bakery and meat products, is influenced by the concentration of proteins and salts, as well as by pH, ionic strength, surface properties, the presence of polar and non-polar amino acids, particle size and ingredient processing history, as reviewed by Kinsella (1976). A high WBC is advantageous when increased viscosity or thickness is desired, whereas it can be seen as a challenge if it negatively affects the techno-functionality of other ingredient components by competing for the available water (Katina et al. 2006; Kinsella 1976). A good OBC is a favorable functionality in, for example, emulsion stabilisation and high-fat food systems, such as processed meats and meat analogues. It is mainly related to the physical entrapment of the oil but may also be affected by the polarity of the amino acid side chains in the proteins (Elleuch et al. 2011; Kinsella 1976).

A substantial amount of literature is available on the functionality of wheat bran, rice bran and barley proteins, whereas only a little is known about the technological functionality of rye proteins. Cereal proteins generally exhibit poor gelling, emulsifying and foaming properties when compared with their animal-based counterparts. A collection of studies describing cereal protein functionality is given in Table 7.

Table 7. A collection of the results of the techno-functional properties of cereal protein concentrates and isolates.

Raw material	Functionalities	Environmental conditions	Main findings	Reference
Rice bran protein isolate	• Minimum gel concentration	• Heating to 95°C and cooling, at pH 4 and 7	• No gels formed (protein content up to 15% tested)	Yeom et al. (2010)
	• Foaming	• pH 5–8, with/without autoclaving	• Foaming capacity increased with increasing pH, stability was the highest at pH 7, autoclaving increased the capacity and lowered stability	
	• Emulsifying	• pH 5–8, with/without autoclaving	• Emulsifying activity and stability increased with increasing pH, the impact of autoclaving was minor	
Proteins extracted from brown rice, white rice and rice bran	• Foaming and emulsifying properties	• pH 3–11, 0.4–2.0% NaCl, 4–20% sucrose	• All the proteins showed improved properties at pH values far from the isoelectric point but they did not differ greatly between each other, foam stability decreased with increasing pH and bran protein formed the most stable foam and the addition of sucrose and NaCl improved emulsifying capacity and stability, respectively	Cao et al. (2009)
Rice bran protein concentrate	• Heat-induced gelation	• Heating to 90°C and cooling	• No gel formed (solid content up to 15% tested), the G' values were low and the greatest increase in G' occurred during cooling	Rafe et al. (2014)
Rice bran protein fractions based on solubility classes (Osborne classes)	• Foaming and emulsifying	• pH 2–12	• Both properties were superior for the albumin and globulin fractions compared with those of prolamins and glutelins, and deviating pH from IP improved the properties	C. Wang et al. (2014)
	• WBC and OBC	• 2 500 r/min, 20 min and 1 000 r/min, 10 min	• 1.1–5.1 g/g and 1.0–3.6 g/g, respectively with variation due to the two different extraction methods applied for fractionating the protein classes	
Rice bran protein concentrates derived from several varieties	• Foaming	• pH 5, 7, 9, 0.5–1.5% NaCl, 5–15% sucrose	• Impact of salt and sugar addition seemed to be variety dependent, deviating pH from IP improved the foaming capacity, the addition of salt improved foaming capacity and stability, sugar addition gave a mixed response for the foaming capacity but greatly improved stability	Chandi and Sogi (2007)
	• Emulsification	• pH 5, 7, 9, 0.5–1.5% NaCl, 5–15% sucrose	• Impacts of pH and salt were variety dependent and sugar improved capacity and stability	
	• WBC and OBC	• 750 × g, 15 min	• 3.9–5.6 g/g and 3.7– 9.2 g/g, respectively	

Wheat bran protein isolate	• Heat-induced gelation	• Boiling (1h) and cooling, water and 1 M NaCl	• No gel formed in water, whereas in 1M NaCl, strong gels formed at 8–14% solid content and very strong gels formed at >16% solid content	Idris et al. (2003)
	• Foaming and emulsifying	• pH 1.5–12 and 0–2.0 M NaCl	• Deviating pH from IP improved emulsifying and foaming capacites, alkaline pH had more impact, emulsion stability increased at an alkaline pH, foam stability was the lowest at IP and salt improved emulsifying and foaming capacities	
	• WBC and OBC	• 2 200 × g, 30 min	• 4.2 g/g and 1.6 g/g, respectively	
Wheat bran protein isolate	• Foaming and emulsification	• 50 mM sodium phosphate buffer (pH 7.4)	• Poor foaming and emulsification stabilities	Arte et al. (2019)
Barley protein isolate	• Foaming properties	• pH 2–11, protein concentration 0.05–1.0%	• Deviating pH from IP and increasing protein concentration increased foam volume and half-life	Yalçin et al. (2008)
Barley protein fractions (hordein, glutelin, pearled grain flour, pearling flour rich in albumins and globulins)	• Foaming	• pH 3, 5 and 8, protein content 0.5% w/v	• The lowest foaming capacities and the highest foam stabilities were at pH 5, the highest foaming capacity and stability were obtained for the hordein and glutelin fraction, respectively, at all the pH values.	Wang et al. (2010)
	• Emulsification	• pH 3, 5 and 8, protein content 0.5% w/v	• In emulsification, similar trends were observed compared to foaming except that there were no major differences in the emulsification capacities of the fractions	
	• WBC and OBC	• 2 000 × g, 30 min, 23°C	• 2.2 g/g (hordein) to 4.2 g/g (glutelin) and 5.2–5.7 g/g, respectively	
Barley protein concentrate	• Emulsion capacity and stability	• pH 2–10	• The lowest capacity and stability were found at pH 6	Bilgi and Çelik (2004)
Defatted barley flour and acid-precipitated barley protein isolate	• Foaming	• pH 7, 10 mg protein/ml	• There was no differences in the foaming properties of the two ingredients	Mohamed et al. (2007)
	• Emulsification	• pH 7, 1 mg protein/ml	• Emulsification properties were slightly better for the isolate than for the flour	

IP: isoelectric point; OBC: oil binding capacity; WBC: water binding capacity.

Understanding the technological functionality of dry-fractionated plant protein ingredients remains scarce in literature. Sosulski and McCurdy (1987) compared the techno-functional properties of air-classified protein-enriched fractions and wet-extracted isolates from faba beans and field peas. Nitrogen solubility index values and foaming properties were considerably higher for the dry-fractionated proteins than for the isolates, whereas the isolates exhibited better oil and water binding properties and no clear differences were observed in the emulsification properties. Interestingly, the raw material flours showed the highest nitrogen solubility indexes and the decrease in solubility for the air-classified samples was postulated to result from heat generation during grinding or improper dispersability of the fine protein powders (Sosulski and McCurdy 1987). An air-classified protein concentrate from lupine was reported to exhibit considerably higher foam stability than an isolate produced by wet processing including a heating step (Pelgrom et al. 2014). A recent study by Vogelsang-O'Dwyer et al. (2020) demonstrated that a protein-enriched faba bean ingredient produced by dry fractionation exhibited higher protein solubility, foaming capacity and gelling ability compared with a wet-extracted faba bean protein isolate. Lundgren (2011) and Ranhotra et al. (1992) evaluated the baking performance of protein-enriched wheat flours and observed the increased water absorption of the protein flours. This was postulated to be due to the increased protein and damaged starch contents resulting in sticky doughs (Lundgren 2011). The breads prepared using the protein-enriched flours were larger in volume but had a darker crust colour (Lundgren 2011).

2.3.3 TECHNOLOGICAL FUNCTIONALITY OF STARCH AND DIETARY FIBRE

DF and starch content affect many of the technological functionalities of cereal ingredients. Moreover, the interactions between the multiple components in flours and concentrates may affect the overall ingredient functionalities. The main component in whole grains is starch, which is usually also present in large quantities in bran and other cereal side stream fractions. The most characteristic starch property is its behaviour during heating and cooling. When solutions of native starch are heated in water, the starch granules swell and their crystalline structure is lost, which is called gelatinisation (Cornejo-Ramírez et al. 2018; Tester and Morrison 1990). Partly simultaneously, pasting takes place, during which the viscosity of the starch-water suspension increases. After further heating, the leaching of the linear amylose component of starch and the disruption of starch granules occur, and a rapid drop in solution viscosity is observed when forces (e.g. mixing forces) are applied. After cooling down, the amylose and amylopectin molecules realign, viscosity increases and eventually the starch retrogradates (S. Wang et al. 2015). In addition to the behaviour of the starch in heating, the structural elements of starch have an impact on the technological functionality. For example, the ratio

between amylose and amylopectin molecules and the presence of damaged and resistant starches affect the ingredient properties. The impact of damaged starch can be seen as highly relevant when considering dry processing as fine milling is known to induce the formation of damaged starch (Drakos et al. 2017a; Tester 1997). Damaged starch has been shown to bind water more efficiently than native starch as starch becomes more prone to hydration and surface area increases when damaged (Berton et al. 2002; Drakos et al. 2017a; Pelgrom et al. 2013). Furthermore, damaged starch is more prone to hydrolysis by amylolytic enzymes (Barrera et al. 2016).

The main non-starch polysaccharides in cereal grains include arabinoxylan, β-glucan, cellulose and pectin. In addition, variable amounts of other polysaccharides, such as heteroxylans, xyloglucan and heteromannans, may be present, as reviewed in Burton and Fincher (2014). The ratios between different fibrous components, as well as soluble and insoluble fibres, are specific to each cereal species. The most remarkable fibre properties affecting the technological functionality are linked with their solubility and insolubility. As reviewed by Elleuch et al. (2011), SDF, such as β-glucan, arabinoxylan and pectin, increases solution viscosity, forms gels and even acts as an emulsifier, whereas IDF, such as arabinoxylan, cellulose and lignin, swells and binds water and oil. Thus, the fibrous components may improve the stability of food products, for example, by retarding syneresis, stabilising dispersions due to increased viscosity and modifying the structure. In addition to the behaviour of DF in water, some remarkable properties are observed in modified aqueous environments. For example, a heated low-methoxyl pectin solution is known to gel, especially at slightly alkaline conditions, during cooling in the presence of calcium due to calcium-bridge-mediated pectin network formation (Yang et al. 2018). A wet-extracted rice bran DF preparation has been shown to have a higher OBC and emulsifying capacity compared with a commercial fibre preparation (Abdul-Hamid and Luan 2000). A comparison of differently produced DF ingredients from defatted rice bran was also performed by Wang et al. (2016) and the authors reported that the dry-fractionated ingredients exhibited comparable, or even higher, swelling, WBC and OBC than the wet-extracted rice bran DF. In regard to sensory attributes, soluble oat bran fibres are perceived to have a more pleasant mouthfeel than insoluble oat bran fibres in high-moisture food applications (Chakraborty et al. 2019).

2.3.4 STRATEGIES TO IMPROVE THE FUNCTIONAL PROPERTIES OF PLANT INGREDIENTS

Due to the low protein solubility and inferior techno-functional properties of plant proteins and ingredients when compared with animal-based proteins, various approaches for cereal protein and ingredient functionalisation have been investigated. The main functionalisation approaches can be divided into physical, hydrothermal and biochemical processing methods. Physical methods include, for example, ultrasound treatment, colloid or dry milling and

microfluidisation. High-intensity, low-frequency (16–100 kHz) ultrasound treatment is based on compression and rarefaction cycles formed in the liquid media as a result of mechanical acoustic waves. These cycles induce the enlargening of small gas bubbles in the liquid and, when reaching a critical size, they collapse (i.e. cavitate), leading to rapid heat and pressure shocks, mixing, microstreaming currents and turbulence, for example (Kadam et al. 2015; Knorr et al. 2004; Leighton 1998; Soria and Villamiel 2010). Sonication has been extensively studied in legume protein functionalisation and improvements, especially in the protein solubility of soy protein (Hu et al. 2015; Jambrak et al. 2009; Lee et al. 2016; Tang et al. 2009; Yildiz et al. 2017), pea protein (Jiang et al. 2017), black bean protein (Jiang et al. 2014), millet protein (Nazari et al. 2018), faba bean protein (Martínez-Velasco et al. 2018) and canola protein (Flores-Jiménez et al. 2019); the foaming properties of pea protein (Xiong et al. 2018) and canola protein (Flores-Jiménez et al. 2019); and the emulsification, gelation and oil absorption properties of canola protein (Flores-Jiménez et al. 2019), are reported. Moreover, starch modifications, such as the formation of nano-sized particles (Bel Haaj et al. 2013) and increased water absoption properties (Sujka and Jamroz 2013), have been reached via intensive ultrasonication.

Enzymatic treatments with carbohydrate or protein hydrolysing enzymes have improved protein solubilisation from various cereal matrices due to bran protein liberation (Arte et al. 2019, 2016; Coda et al. 2014b; Nordlund et al. 2013b) or reduction in the molecular weight of the proteins (Nisov et al. 2020; Paraman et al. 2007; Xu et al. 2016), respectively. In addition, improvements in functionality may be obtained via enzymatic hydrolysis, cross-linking or deamidation (reviewed by Day 2013). Furthermore, lactic acid fermentation has improved cereal protein functionality and properties (Arte et al. 2019; Coda et al. 2014a). In addition to enzymatic degradation of the macromolecules in plants, enzyme-aided modifications via degradation of the minor compound, phytic acid, has been achieved. Phytase treatments have mainly targeted the improved nutritional quality of feed materials via the reduction of protein and mineral binding (as reviewed by Gupta et al. 2013). Additionally, phytase treatment has improved protein solubilisation from rice bran during wet protein isolation (Wang et al. 1999) and improved the solubility of rice pollards (Kies et al. 2006).

In regard to chemical treatments, one alternative for modifying plant protein functionality is to apply the pH-shifting approach wherein the proteins are exposed to extreme alkaline or acidic conditions, after which they are readjusted to neutral conditions. Shifting may induce changes in the tertiary structure via the partial unfolding and refolding of the protein, which may also be referred to as a molten globule state (Christensen and Pain 1991; Hirose 1993; Jiang et al. 2018). In literature, pH shifting to high acidic and alkaline conditions has improved the protein solubility, surface hydrophobicity and emulsifying properties of soy protein isolate (Jiang et al. 2010, 2009) and

improved the solubility of pea protein isolate (Jiang et al. 2017). However, studies concerning the pH shifting of cereal proteins are scarce.

3 AIMS OF THE STUDY

Cereal grain processing generates annually vast amounts of side streams. Valorisation of those side streams into food use is regarded a promising approach from both sustainability and food security points of view. Traditionally, cereal ingredient production aims at manufacturing pure component fractions, such as proteins, DF and starch. However, less-refined multicomponent ingredients can offer feasible solutions by providing opportunities to exploit both the techno-functional and nutritional properties of all the components present in the ingredients. Dry fractionation offers a sustainable and energy-efficient production method for preparing such hybrid ingredients that are enriched in protein but also contain valuable amounts of other, nutritionally and techno-functionally important components, such as DF and starch. Therefore, the main aim of this work was to identify the underlying principles involved in the dry fractionation of cereal side streams in order to produce protein-enriched hybrid ingredients, and the factors affecting the technological functionality and applicability of the hybrid ingredients in relevant food matrices.

The specific objectives were:

1. To develop dry fractionation technologies combined with suitable pre-treatments for protein enrichment from cereal side streams deriving from rice, wheat, rye and barley (I, II, III)

2. To investigate the techno-functional properties of the dry-fractionated, protein-enriched cereal fractions and to understand the role of starch, fibre and other components specific to each raw material in terms of functional properties (I, II, III)

3. To examine modification of the techno-functional properties of the protein-enriched materials for improved performance in high-moisture food systems; the treatments investigated for altering the technological functionality of cereal ingredients included (i) enzymatic hydrolysis of the components influencing the ingredient performance, in this case, phytic acid (IV), and (ii) physical processing, in this case, ultrasound treatment with and without pH shifting for particle size reduction and structural modification (V)

4 MATERIALS AND METHODS

This section presents a general outline of the materials and methodology applied in this work. More detailed descriptions can be found in the original publications (Publications I–V).

4.1 RAW MATERIALS, FLOW AID AND ENZYME

The cereal raw materials utilised in this work and their compositions are listed in Table 8. In Publication III, a food-grade flow aid Aerosil® 200F, composed of fumed silica particles (Evonik Industries AG, Essen, Germany), was utilised. The food-grade microbial phytase from *Apergillus niger* that was utilised in Publication IV was purchased from Ultra Bio-Logics Inc. (Quebec, Canada). The enzyme activity was, according to the manufacturer, 1 500 U/g and optimum conditions for the enzyme were pH 4.5–6 and 45–55°C. Potential protease side activity in the enzyme preparation was excluded by the analysis described by Matsubara and Nishimura (1958).

4.2 PRE-TREATMENTS PRIOR TO DRY FRACTIONATION

In this work, different pre-treatments were applied for all the raw materials prior to dry fractionation and selection of the pre-treatment was made based on raw material properties and the targeted impact of the treatment on the composition or behaviour of the material in further dry processing. The pre-treatments studied in this work are shown in Figure 5.

Rice bran was defatted as described earlier for rapeseed press cake (Rommi et al. 2015a) with SC-CO_2 extraction using a Nova Swiss extraction vessel (Nova Werke AG, Effretikon, Switzerland) with a Chematur Ecoplanning compressor (Chematur Engineering Ltd., Pori, Finland) under extraction pressure of 29.5–30.5 MPa (I, IV). The temperature was 40 and 48°C in extraction and separation containers, respectively, and five kilograms of CO_2 circulated in the equipment during each extraction. Wheat and rye brans were dried in an oven for 48 h at 40°C to reach moisture contents of 4.9% (rye bran) and 5.2% (wheat bran) prior to dry milling (II). Prior to the air classification of the barley endosperm fraction, the raw material was mixed with 0.5% (w/w) Aerosil® 200F fumed silica particles (III, V).

Table 8. The raw materials used in dry fractionation.

Raw material	Description	Supplier	Protein (% dm)	Starch (% dm)	Insoluble dietary fibre (% dm)	Soluble dietary fibre (% dm)	Lipids (% dm)	Ash (% dm)	Publication
Rice bran	Non-heat-treated rice bran of the Indica variety (*Oryza sativa* L. ssp. Indica)	Südzucker AG, rice starch factories, Italy	15.5[a]	19.7[a]	25.6[a]	5.4[a]	21.8	8.8[a]	I, IV
Wheat bran	Wheat bran (V6200), commercial sample	Fazer, Fazer Mills, Lahti, Finland	16.4	15.9	42.4	9.2	na	6.0	II
Rye bran	Rye bran (R4500), commercial sample	Fazer, Fazer Mills, Lahti, Finland	14.7	40.1	21.3	11.8	na	4.0	II
Barley endosperm fraction	Dry processing of industrial barley to obtain barley grits (27%); pin disc milling and sieving the grits (<150 µm, 40%) results in the barley endosperm fraction	Altia Corporation, Finland	8.3	80.0	1.6	1.8	1.2	0.7	III, V

[a] values calculated based on the mass balances of the SC-CO_2 fat extraction step.
na: not analysed.

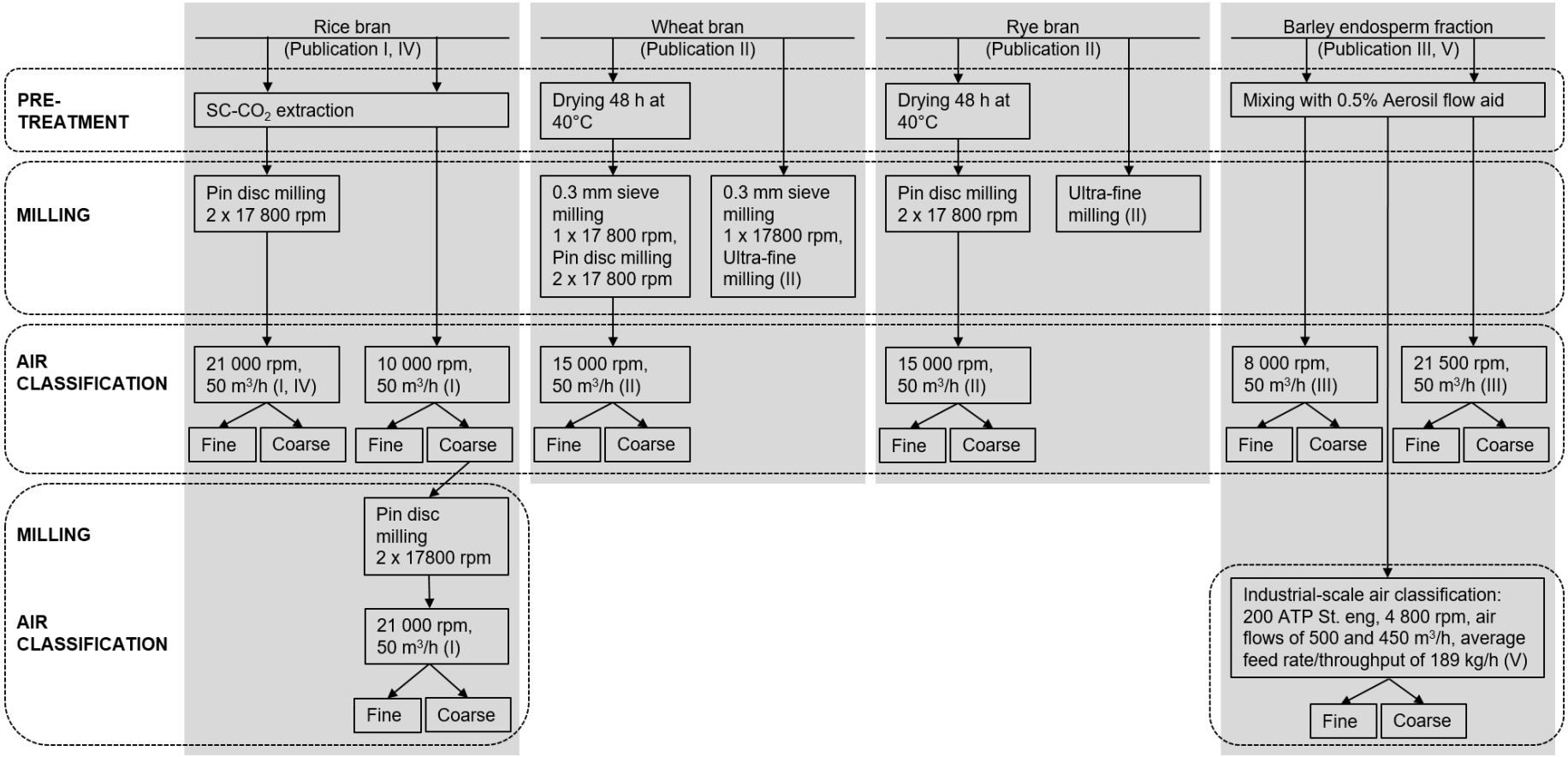

Figure 5. The pre-treatment and dry fractionation processes applied in different publications. SC-CO_2: supercritical carbon dioxide.

4.3 DRY FRACTIONATION

4.3.1 MILLING

Milling procedures for each of the different cereal raw materials were selected based on their known special features, such as composition and resistance to particle size reduction, and based on targeted analysis of the milled materials.

In Publication II, the dried wheat bran was first milled using a 0.3 mm sieve with a 100 UPZ fine impact mill (Hosokawa Alpine AG, Augsburg, Germany) at a rotor speed of 17 800 rpm due to the initially large particle size of wheat bran compared with the other raw materials. As a final step prior to air classification, SC-CO_2-extracted rice bran (I, IV), dried and sieve-milled wheat bran (II) and dried rye bran (II) were milled twice using a 100 UPZ pin disc mill (Hosokawa Alpine AG, Augsburg, Germany). In addition, when aiming at understanding the impact of bran particle size reduction and comparing brans and air-classified fractions (Section 4.3.2) with rather similar particle sizes, ultra-fine milling was applied. In ultra-fine milling, pre-sieve-milled, non-dried wheat bran and non-pre-milled, non-dried rye bran were milled using a Masuko Sanqyo decompression air-flow-type ultra-fine micronizer (Ceren Miller Dau MKCL8-15J DAU, Masuko Sangyo, Japan) with the rotor speed of 7 600 rpm and trituration time of 1.5 min to obtain ultra-finely milled wheat and rye bran ingredients (II).

4.3.2 AIR CLASSIFICATION

Air classifications were performed at pilot scale using a 50 ATP classifier (Hosokawa Alpine, Augsburg, Germany) (I, II, III, IV). Pin disc-milled SC-CO_2-extracted rice bran was air classified using the air classifier wheel speed of 21 000 rpm and the air flow of 50 m^3/h (I, IV), whereas pin disc-milled wheat and rye brans were air classified using the air classifier wheel speed of 15 000 rpm and the air flow of 50 m^3/h (II), as depicted in Figure 5. In addition, the defatted rice bran was air classified without a pre-milling step at 10 000 rpm and 50 m^3/h (I). The resulting non-milled fine fraction from rice bran was utilised in further analytics, whereas the coarse fraction was further milled (2 x 17 800 rpm using a pin disc mill) and air classified (50 ATP classifier, 21 000 rpm, 50 m^3/h) in order to liberate intact proteins from the aleurone cells and to remove fibrous cell wall structures from the second-step fine fraction (I).

In Publication III, the barley endosperm fraction mixed with 0.5% (w/w) Aerosil 200F was air classified using various air classifier wheel speeds (4 000–21 500 rpm) at the air flow rate of 50 m^3/h. After optimisation of the air classification parameters, the two most promising fine fractions that aimed at the highest protein content and high PSE with considerably increased protein content when compared with the raw material, were 21 500 rpm and 8 000

rpm, respectively. Those two fractions were further evaluated for their physical, compositional and microstructural properties. Barley endosperm fraction mixed with 0.5% (w/w) Aerosil 200F was additionally air classified at an industrial scale (V). At the industrial scale, a Hosokawa Alpine 200 ATP St. eng air classifier (Hosokawa Alpine, Augsburg, Germany) was utilised and operated at the speed of 4 800 rpm using central and tangential air flows of 500 and 450 m^3/h, respectively, and an average feed rate/throughput of 189 kg/h (V). The fine fractions from all the air classifications were defined as protein-enriched fractions (I, II, III, IV, V).

Raw material performance in the air classifications was evaluated by calculating mass yield and (total) PSE according to Equations 1–3 as follows:

$$\text{Mass yield (\% dm)} = \frac{\text{dry weight of fraction (g)}}{\text{dry weight of raw material (g)}} \times 100\%, \quad (1)$$

$$\text{Protein separation efficiency (PSE \% dm)} = \frac{\text{dry weight of fraction (g)} \times \text{protein content of fraction (\% dm)}}{\text{dry weight of raw material (g)} \times \text{protein content of raw material (\% dm)}} \times 100\%, \quad (2)$$

$$\text{Total PSE (\% dm)} = \frac{\text{PSE of previous processing step (\% dm)}}{100} \times \frac{\text{PSE of the fraction in question (\% dm)}}{100} \times 100\%. \quad (3)$$

The selection of air classification parameters was done individually for each raw material based on pre-trials (data not shown) that aimed at fine fraction mass yields of >5% or preferably >10%.

4.4 FUNCTIONALISATION

Protein-rich ingredients (IV, V) were treated with different methods that aimed at improving their technological functionalities relevant in high-moisture foods.

4.4.1 PHYTASE TREATMENT

In Publication IV, the protein-enriched rice bran fraction produced by air classification was treated with a phytase to observe the impact of phytic acid degradation on protein properties and heat-induced gelation ability of the ingredient.

The effect on protein solubility was investigated by dispersing the protein-enriched rice bran fraction to 1% protein concentration using Milli-Q water and adjusting the pH to 5, followed by the addition of phytase (100 U/g dm) and incubation for 2 h at pH 5 and 50°C. After incubation, the sample was cooled to room temperature and adjusted to pH 6.7 (i.e. the native pH of the flour). The non-heat-treated sample for protein surface hydrophobicity determination was drawn at this stage. After pH adjustment, the sample was heat treated in

a boiling water bath (sample temperature >92°C for 5 min) with manual shaking for enzyme inactivation. After heat treatment, the sample was divided into six aliquots and the pH of the aliquots was adjusted (pH 2, 4, 6, 6.7, 8 and 10), the samples were centrifuged (10 000 × g, 10 min, 20°C) and the supernatant was collected for protein solubility and protein surface hydrophobicity (a heat-treated sample at pH 6.7) determination (see Section 4.5.2). A control sample was prepared that was similar to the phytase-treated sample but no enzyme was added to control dispersion.

In gelation trials, the phytase treatment was performed at 14% dry matter content (3.3 and 3% protein and total DF, respectively, in order to fulfil the nutritional claim 'source of fibre'). After overnight hydration at 6°C, the dispersion containing 0.01% of NaN_3 as a preservative was adjusted to pH 5 and treated with phytase (100 U/g dm) for 2 h at pH 5 and 50°C. At the end of the incubation, the sample was adjusted to pH 5, 6.7 or 8 for the gelation experiments (see Section 4.5.6). Control samples were prepared in a similar way to the phytase-treated samples but without added enzyme. The enzyme was not separately inactivated right after the enzyme treatment since the samples were directly tested for their heat-induced gelation ability, and the heating included in this step was presumed to result in enzyme inactivation.

4.4.2 ULTRASOUND TREATMENT

In Publication V, the protein-enriched barley fraction was compared with a barley protein isolate that was prepared from the protein-enriched barley fraction according to a protocol adapted from Wang et al. (2010). In brief, alkaline extraction included the pH adjustment of a 10% (w/w) dispersion to pH 11 and centrifugation after 30 min (10 000 × g, 15 min, 20°C) in order to collect the soluble proteins into the supernatant. After adjusting the pH to 5, followed by 30 min stirring, the precipitated proteins were separated by centrifugation (10 000 × g, 15 min, 20 °C). The solid residue was freeze-dried and milled with a laboratory-scale ultra-centrifugal mill, ZM200, equipped with a 0.5 mm sieve (Retsch, Hann, Germany).

For functionalisation, both the protein-enriched barley fraction and the barley protein isolate were treated with ultrasound using a procedure adapted from Jiang et al. (2017) in order to investigate the impact of ultrasound on the physicochemical properties of the ingredients. The raw materials were dispersed into Milli-Q water at 1.5% (w/w) and mixed using a magnetic stirrer for 30 min, followed by pH adjustment to pH 3, 7 or 9 and pH readjustment after 30 min if necessary. Control samples were under magnetic stirring all through the treatment duration whereas the ultrasound-treated samples were homogenised with a VC 750 ultrasonic processor (Sonics & Materials, Inc., Newtown, CT, USA) equipped with a 13 mm probe. Ultrasonication was carried out twice for 2.5 min at 20 kHz and with the amplitude of 100%. In order to avoid excessive heating, samples were kept in an iced water bath, both in between the two treatments and during ultrasonication. After

sonication, the dispersions were kept under magnetic stirring for 60 min, followed by pH adjustment of one aliquot to pH 7 with the other aliquot remaining at pH 3 or pH 9. The samples were used as fresh, directly after the treatments.

4.5 ANALYTICAL METHODS

4.5.1 COMPOSITION, MICROSTRUCTURE AND PARTICLE SIZE

The biochemical composition of the raw materials and air-classified fractions was determined in order to elucidate the impact of processing on the macronutrients and their fractionation. Total nitrogen content was determined with the Kjeldahl method, according to the AOAC method 2001.11 (Thiex et al. 2002), and converted to protein content using nitrogen-to-protein conversion factors of 5.95 for rice proteins (Juliano and Bechtel 1985) (I, IV) and 6.25 for wheat, rye and barley proteins (II, III, V). Total starch content was analysed either according to the AACC 76–13.01 method, using the Megazyme total starch assay kit (I, II, IV, V) or using Ewers polarimetric method (ISO 10520:1997 1997) (III). Damaged starch was quantified according to the AACC 76-31.01 method with the Megazyme starch damage assay kit (II). In Publications I, III and IV, the content of total DF was determined using the enzymatic-gravimetric AOAC method 991.43 (AOAC 1995), which differentiates the high molecular weight SDF and IDF. In Publication II, the enzymatic-gravimetric AOAC method 2011.25 according to McCleary et al. (2012) was utilised, where the total DF content was obtained as a sum of high molecular weight insoluble dietary fibre (HMWIDF), high molecular weight soluble dietary fibre (HMWSDF) and low molecular weight soluble dietary fibre (LMWSDF). Ash content was determined gravimetrically after combustion at 550°C (I, II, III, IV). Phytic acid content was determined using the colorimetric method described by Latta and Eskin (1980) with slight modifications following Vaintraub and Lapteva (1988) (I, II, IV). Lipid content was determined gravimetrically after 5 h Soxhlet extraction with heptane (I, III).

For the microscopy analyses, rice bran (I) and barley endosperm (III) samples were prepared as described in Holopainen-Mantila et al. (2013). The samples were stained with Calcofluor White and Acid Fuchsin, according to Andersson et al. (2011), and examined under a fluorescence microscope to visualise intact cell wall glucans as blue and proteins as red. Due to autofluorescence, pericarp structures were observed as brownish yellow structures (I) whereas starch was unstained and appeared black (I, III). Additionally, in Publication III the samples were stained with Light Green SF and diluted Lugol's iodine solution, as also described in Andersson et al. (2011), in order to visualise proteins as green, the amylose component of starch as blue and amylopectin as brown.

The volume-based particle size distributions of the powdered cereal samples were determined by laser light diffraction (750 nm) using a Beckman Coulter LS 230 (Beckman Coulter Inc., Brea, CA, USA) (I, II, III, unpublished data related to V). The samples were dispersed in ethanol and analysed using the liquid module and ethanol as a carrier and using refractive indices 1.36 (ethanol) and 1.50 (starch) for the media and sample, respectively. In Publication V, the particle size distributions of the ultrasound-treated and control samples were analysed by the Beckman Coulter LS 230, this time using a liquid module with filtered Milli-Q water as the carrier and refractive indices of 1.33 and 1.50 for the dispersant and the particles, respectively.

4.5.2 PROTEIN SOLUBILITY, PROTEIN PROFILE AND SURFACE HYDROPHOBICITY

Protein solubility was determined in order to reveal changes in protein properties as a result of dry processing (I, II, III), bioprocessing with phytase (IV) or ultrasound treatment (V). Protein solubility (%) was expressed as the amount of soluble protein (mg/ml) remaining in the supernatant after centrifugation (10 000 × g) in relation to the protein content (mg/ml) of the original aqueous sample dispersion. The protein solubility of the rice, wheat and rye bran raw materials and air-classified fractions was determined by dispersing the samples in a 2% (w/w) protein concentration and adjusting to pH 5, 6.7–6.8 ± 0.2, and 8, followed by mixing and readjustment of the pH if needed at 30 and 60 min (I, II). In regard to phytase-treated samples, the protein content of the dispersion was 1% (w/w) and the pH values studied were pH 2, 4, 6, 6.7, 8 and 10, as described in Section 4.4.1. In this entity, lower protein concentration was utilised since enzyme inactivation by heating would have resulted in sample gelation at higher concentrations, thus hindering protein solubility determination (IV). The protein solubility of the protein-enriched barley endosperm fraction was determined from water dispersions at 5% dry matter content, and the dispersions were adjusted to a pH range of 3–11, followed by mixing and readjustment of the pH if needed at 60 and 120 min (III). Finally, the samples were centrifuged (10 000 × g, 15 min (I, II, III) or 10 min (IV), 20°C) and the total nitrogen concentration of the supernatant was determined with the Kjeldahl method and converted to protein concentration using nitrogen-to-protein conversion factors of 5.95 for rice proteins (Juliano and Bechtel 1985) (I, IV) and 6.25 for wheat, rye and barley proteins (II, III). The impact of ultrasound treatment and/or pH shifting on the protein solubility of the protein-enriched barley endosperm fraction and barley protein isolate (V) was evaluated by quantifying the protein concentration of the supernatants separated from the 1.5% (w/w) dispersions by centrifugation (10 000 × g, 10 min, 20°C). Quantification was performed using a commercial kit (DC Protein Assay, Bio-Rad, Hercules, CA, USA), which is based on the Lowry protein assay (Lowry et al. 1951). Bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard protein.

Protein profiles of the water mixtures of rice, wheat and rye bran and barley endosperm samples were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970) under both reducing conditions (I, II, V) and non-reducing conditions (V). Water dispersions of raw materials or protein-enriched fractions were mixed with the sample buffer (20% glycerol, 4% SDS, and 0.02% bromophenol blue in a 0.1 M Tris-HCl pH 6.8 buffer, with or without 10% β-mercaptoethanol under reducing and non-reducing conditions, respectively), followed by heating at 98°C for 5 min, and were loaded with 30 µg protein on a Criterion TGX, Stain-Free Precast 4–20% Tris-HCl gradient gel (Bio-Rad, Hercules, CA, USA). The protein bands were visualised with Criterion Stain Free Imager and examined using Image Lab software (Bio-Rad).

The protein surface hydrophobicity of the protein-enriched rice bran sample (IV) was analysed as described in Hayakawa and Nakai (1985) with slight modifications using 1-anilino-8-naphthalene sulfonate (ANS) as the hydrophobic probe. The heat-treated and non-heat-treated phytase-treated and control sample supernatants at pH 6.7 (Section 4.4.1) were diluted to concentrations between 0.02 and 1.0 mg protein/ml using a 0.05 M phosphate buffer at pH 6.7 and mixed with 0.8 mM ANS at a ratio of 1:1. Fluorescence intensity was measured after 5 min incubation in the dark using a Varioskan™ LUX multimode microplate reader (Thermo Scientific) at excitation and emission wavelengths of 390 and 470 nm, respectively. The slope of the linear regression of absorbance plotted against protein concentration was defined as the surface hydrophobicity.

4.5.3 DISPERSION STABILITY, EMULSIFICATION AND FOAMING

The dispersion stability of the bran ingredients (i.e. the stability of the particles in aqueous dispersions prepared under magnetic mixing) was analysed by visual observation of the sedimentation of a 4% (w/w) dispersion as a function of time (I, II). Likewise, the colloidal stability of ultrasonicated (and control) barley protein ingredients (V) (i.e. the stability of the particles in colloidal dispersions homogenised by ultrasound treatment) was analysed by visual observation of the sedimentation of a 1.5% (w/w) dispersion as a function of time. The emulsification ability of wheat and rye bran protein-enriched and ultra-finely milled ingredients in Publication II was evaluated at 10% w/w dispersion with and without 10% rapeseed oil added. Emulsions were prepared by homogenising the aqueous ingredient dispersion with and without oil using a VC 750 ultrasonic processor (Sonics & Materials, Inc., Newtown, CT, USA) equipped with a 13 mm probe. Sonication was carried out for 3 min at 20 kHz and with an amplitude of 70%, and the samples were immersed in an iced water bath to prevent samples from overheating. The colloidal stability of the emulsions was analysed by visual observation of the emulsions as a function of time. Particle size analysis of the emulsions was carried out in filtered Milli-Q water with a Mastersizer 3 000 Hydro (Malvern Analytical,

Worcestershire, UK) using refractive indices of 1.33 and 1.53 for the dispersant and the particles, respectively. In Publication III, the foaming properties of the protein-enriched barley fraction were studied by mixing 25 ml of 4% (w/w) dispersion for 1 min with a battery-operated whisker (AeroLatte AL-V1-SS Chef Kitchen Whisk, United Kingdom).

4.5.4 WATER AND OIL BINDING CAPACITIES

WBC of a barley endosperm fraction, as well as differently milled raw materials and fractions from wheat and rye brans, was defined as the amount of water (g) retained by the sample (g) by mixing 1 g of the sample with 10 ml of distilled water, followed by incubation for 30 min (vortexing every 10 min). After incubation, the supernatant was removed by centrifugation (2 000 × g, 10 min) (Quinn and Paton 1979) and the pellet was weighed. For the same samples, OBC, defined as the amount of oil (g) retained per solid (g), according to Lin et al. (1974), was analysed by dispersing 100 mg of the sample with 1 g of sunflower oil and incubating for 30 min (vortexing every 10 min). After incubation, the supernatant was removed by centrifugation (3 000 × g, 10 min) and the pellet was weighed.

4.5.5 PASTING PROPERTIES

The pasting properties of the barley endosperm, as well as the wheat and rye bran samples, were analysed with a Rapid Visco Analyser (RVA) (Newport Scientific Pty Ltd., Warriewood, NSW, Australia) using the standard Newport Scientific method 1 (II, III).

4.5.6 GELATION AND GEL CHARACTERISATION

In Publication IV, phytase treatment of a protein-enriched rice bran fraction was carried out as described in Section 4.4.1 and its effect on the heat-induced gelation of the fraction was studied. The gelation of the samples at pH 5, 6.7 and 8 was carried out in a rheometer (DHR-2 hybrid, TA-Instruments, Crawley, UK) equipped with a plate–plate geometry using temperature gap compensation. A solvent trap with rapeseed oil was used to prevent evaporation. Gel formation was followed during heating the samples within the geometry from 25 to 95°C at a rate of 2°C/min followed by a hold at 95°C for 5 min, and cooling to 25°C (2°C/min) and a further hold at 25°C for 15 min. The strain and frequency parameters used during the temperature sweep were 0.1% and 0.1 Hz, respectively, and were measured to be within the linear viscoelastic region. The final gel characteristics were analysed by performing a strain sweep from 0.01 to 100% (0.1 Hz) and determining the yield strain (%) at 10% decrease in the average G' from its plateau region. A portion of the same samples that were used for gel formation went through exactly the

same heating and cooling cycle in Eppendorf tubes placed in a manually controlled heating block in order to analyse the WHC. After heating, cooling and overnight storage at 6°C, the sampes were centrifuged (3 000 × g, 10 min, 6°C). The WHC (%) was defined based on the share of the difference between the gel mass and expelled liquid mass in relation to the gel mass, according to Ercili-Cura et al. (2013). The gel microstructures were examined with a confocal laser scanning microscope. Calcofluor White (0.1 ppm) and Rhodamin B (10 ppm) were added to samples before heat-induced gelation to stain the glucan-containing cell wall structures and the proteins, respectively, in the gel matrix. After gelation, the stained gels were spooned onto microscopy slides equipped with an adhesive isolator, forming wells of 9.0 mm in diameter and 1.0 mm in depth that were further protected with a cover glass and examined after overnight storage at 6°C.

4.6 STATISTICAL ANALYSIS

Statistical analysis of protein content, protein solubility (I), protein solubility, WBC, OBC (II), mass yields, protein separation efficiencies, protein contents (III), WHC, surface hydrophobicity (IV) and protein solubility (V) was performed with SPSS Statistics software (versions 24, 25 and 26, IBM, Armonk, NY, USA) using one-way analysis of variance (ANOVA). The level of significance was set at $p<0.05$ and was assessed by Tukey's post hoc test.

4.7 OVERVIEW OF THE EXPERIMENTAL RESEARCH

The work consisted of dry fractionation, a functionality assessment and the functionalisation of cereal side streams, and the main processing steps and analytics utilised in each publication are visualised in Figure 6.

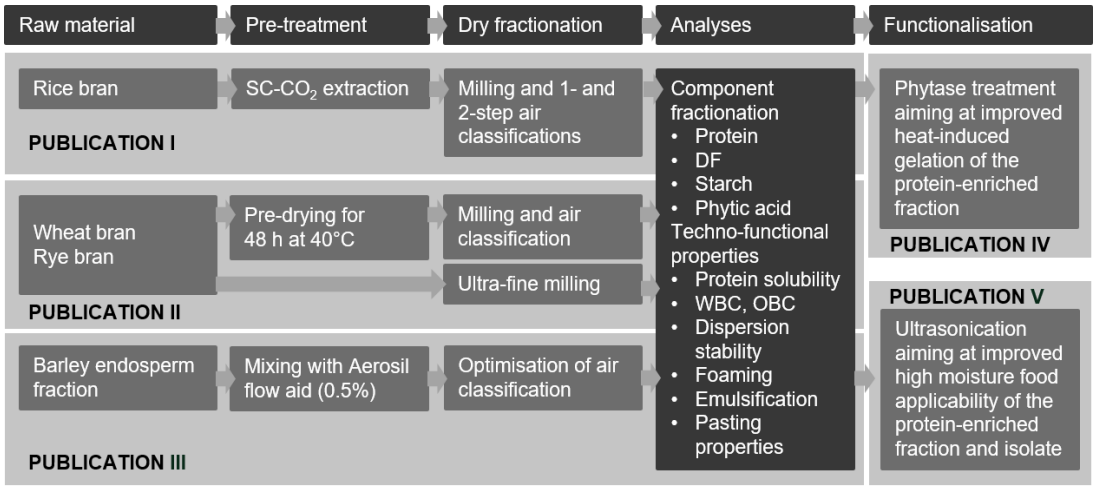

Figure 6. An overview of the experimental research conducted in this thesis. DF: dietary fibre; WBC: water binding capacity; OBC: oil binding capacity.

5 RESULTS

5.1 DRY FRACTIONATION

5.1.1 COMPOSITION AND STRUCTURE OF RAW MATERIALS BEFORE AND AFTER PRE-PROCESSING (I, II, III)

Rice bran (I, IV), wheat bran (II), rye bran (II) and a barley endosperm fraction (III, V) were pre-processed by defatting (I, IV), drying (II) or mixing with a flow aid (III, V), with or without milling prior to dry fractionation that aimed at protein enrichment (Figure 5). Before protein enrichment, the biochemical composition of the raw materials (I, II, III) and their microstructure (I, III) were studied, and the impact of pre-treatments on the raw material properties were elucidated.

The microstructure of the non-heat-treated and full-fat rice bran raw material (Figure 7a) revealed the presence of both intact and disintegrated cell wall structures (I). Protein had partially leaked out from the aleurone cell structures and only some intact aleurone cells were present. The bran sample also contained long pericarp stuctures, some of which were attached to aleurone cell layers. In addition to outer grain layers, some large particles deriving from starchy endosperm and germ were detected. Since the high oil content of the native rice bran (21.8%; Table 8) prevented dry milling of the full-fat bran, SC-CO_2 extraction was applied for oil removal. The fat extraction decreased the oil content of rice bran to 3.2% and resulted in a concurrent increase in protein content from 15.5 (Table 8) to 18.5% (Table 9, the grey background). In the SC-CO_2-extracted bran, the starch content was 23.5% and DF content was 37.0%, of which 6.5% was soluble and 30.5% was insoluble (Table 1 in I). Moreover, the phytic acid content of the rice bran was 8.7%. In addition to modification of the biochemical composition, defatting allowed considerable particle size reduction during pin disc milling (from 339 to 62 µm) and improved the behaviour of the material during air classification, which was not possible for the full-fat bran. Wheat bran (II) contained a higher amount of DF (51.7%; Table 1 in II) and less starch (15.9%) compared with the defatted rice bran (Table 9, the grey background). On the contrary, rye bran (II) exhibited a similar DF level (33.1%; Table 1 in II) with rice bran whereas the starch content was considerably higher (40.1%; Table 9, the grey background). The phytic acid content of wheat and rye brans (4.7 and 2.1%, respectively) was lower than that of rice bran.

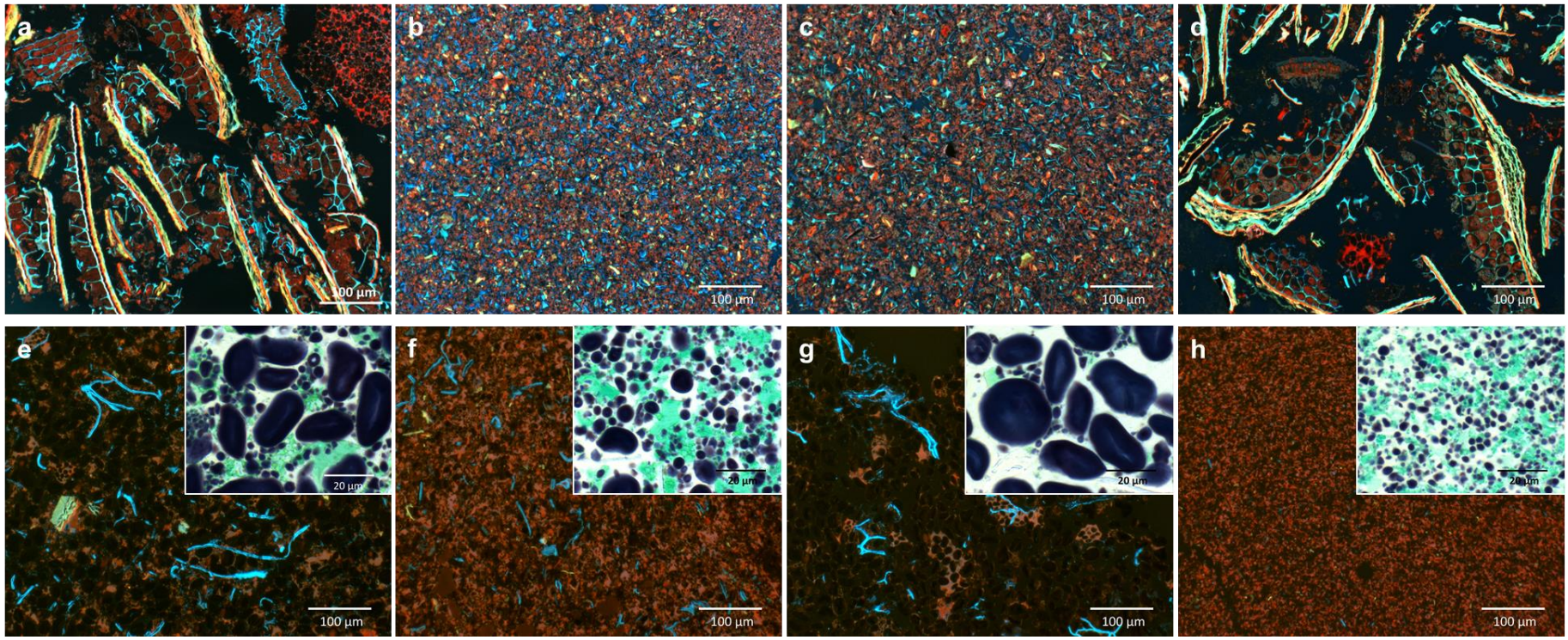

Figure 7. The impact of dry fractionation on the microstructure of rice bran (I) and the barley endosperm fraction (III). In the main images, proteins are shown as red, cell wall glucans as blue and pericarp structures are yellowish, whereas starch is unstained and appears as black. In the upper corner images of the lower row, protein is stained green and starch violet. In the upper row: rice bran (a) full-fat raw material, (b) the fine fraction from the one-step air classification (21 000 rpm), (c) the fine fraction from the first step of the two-step air classification (10 000 rpm), (d) the coarse fraction from the first step of two-step air classification (10 000 rpm). In the lower row: the barley endosperm fraction (e) raw material, (f) the fine and (g) the coarse fractions produced with the air classifier wheel speed of 8 000 rpm, and (h) the fine fraction produced with the air classifier wheel speed of 21 500 rpm in the presence of 0.5% Aerosil 200F (image courtesy of Dr. Ulla Holopainen-Mantila, VTT Technical Research Centre of Finland).

Table 9. The impact of air classification on the component fractionation and particle size of rice bran (I), wheat bran (II), rye bran (II) and barley endosperm fraction (III, V).

Raw material	Processing prior to final air classification	Final air classification and fraction	Total mass yield (% dm)	Total PSE (% dm)	Protein content (% dm)	SDF: IDF ratio	Starch content (% dm)	Phytic acid content (% dm)	Median particle size (µm)	Publication
Rice bran	1. SC-CO_2 extraction	-	-	-	18.5	0.2	23.5	8.7	339/62 (milled)	I
	1. SC-CO_2 extraction 2. Pin disc milling 2 x 17 800 rpm	21 000 rpm, F	27.2	38.0	25.7	0.5	7.9	21.6	5	
	1. SC-CO_2 extraction	10 000 rpm, F	18.5	19.7	19.7	0.6	12.9	24.5	7	
	1. SC-CO_2 extraction	10 000 rpm, C	77.8	76.7	18.3	0.1	23.4	na	327	
	1. SC-CO_2 extr., 2. AC 10 000 rpm, C 3. Pin disc milling of C, 2 x 17 800 rpm	21 000 rpm, F	13.9	20.2	27.4	0.3	6.8	16.5	6	
Wheat bran	1. Drying 48 h at 40°C 2. 0.3 mm sieve milling 1 x 17 800 rpm 3. Pin disc milling 2 x 17 800 rpm	-	-	-	16.4	0.2	15.9	4.7	131 (milled)	II
	1. Drying 48 h at 40°C 2. 0.3 mm sieve milling 1 x 17 800 rpm 3. Pin disc milling 2 x 17 800 rpm	15 000 rpm, F	9.6	18.0	30.9	0.9	14.2	13.6	9	
Rye bran	1. Drying 48 h at 40°C 2. Pin disc milling 2 x 17 800 rpm	-	-	-	14.7	0.6	40.1	2.1	89 (milled)	
	1. Drying 48 h at 40°C 2. Pin disc milling 2 x 17 800 rpm	15 000 rpm, F	12.9	26.9	30.7	1.8	36.3	4.0	7	
Barley endosperm fraction	1. No treatment	-	-	-	8.3	1.1	80.0	na	18	III
	1. Mixing with 0.5% flow aid	8 000 rpm, F	22.1	59.4	22.3	1.2	64.3	na	7	
	1. Mixing with 0.5% flow aid	21 500 rpm, F	6.4	21.7	28.3	1.0	55.3	na	3	
	1. Mixing with 0.5% flow aid, industrial	4 800 rpm, F	20.6	52.9	24.0	na	59.7	na	5	V

PSE: protein separation efficiency; SDF:IDF: soluble-to-insoluble dietary fibre ratio; SC-CO_2: supercritical carbon dioxide; F: fine fraction; C: coarse fraction; AC: air classification.

Both wheat and rye brans were oven-dried (48 h at 40°C) prior to dry milling, which resulted in the smaller particle size of the dried and milled brans compared with non-dried brans (data not shown). However, due to the resistant bran structures, normal impact and sieve milling processes, despite the applied drying step, were only able to micronise wheat and rye brans to particle sizes of 131 and 89 µm, respectively (Table 9). In order to reach a smaller particle size, comparable to that of the dry-fractionated protein-enriched fractions, ultra-fine milling was utilised and resulted in notably smaller median particle sizes of 19 and 17 µm for wheat and rye bran, respectively (II). In addition to changes in particle size, the ratio between SDF and IDF increased when ultra-fine milling was employed instead of pin disc milling (Table 1 in II). Likewise, the amount of damaged starch was increased in ultra-fine milling (3.4 and 5.0% for wheat and rye brans, respectively) compared with the amounts in the pin disc-milled wheat and rye brans (2.0 and 2.2%, respectively; Table 1 in II).

The barley endosperm fraction (III) contained a high amount of starch (80.0%), whereas the protein and DF contents were 8.3% and 3.4%, respectively (Table 9, the grey background; Table 2 in III). Broken cell wall structures, together with protein clusters, were visible from the fluorescence microscopy images besides the unstained regions representing starch (Figure 7e). Thin sections of samples were additionally stained with Light Green SF and diluted Lugol's iodine solution to detect the distribution of proteins and starch in the sample. The analysis of the raw material revealed the presence of starch granules of varying size embedded in a protein matrix. Prior to air classification, the material was mixed with Aerosil 200F flow aid due to the highly adhesive and clumping nature of the initial raw material, which resulted in material adhesion to the air classification chamber when classified. Due to the fact that the addition level of Aerosil to the raw material remained at only 0.5%, it was not assumed to have a great impact on the total composition and was, therefore, not taken into account in the compositional analyses.

5.1.2 COMPONENT FRACTIONATION IN AIR CLASSIFICATION (I, II, III)

The main target in the air classification of the cereal side stream raw materials was protein enrichment. However, attention was also paid to the fractionation of other components (in particular starch, DF and phytic acid) that presumably affect both technological and nutritional properties of the ingredient. Air classification of all the milled raw materials using air classifier wheel speeds of 8 000–21 500 rpm yielded fine, protein-enriched fractions with median particle sizes ranging from 3 to 9 µm, despite the pre-milling steps applied (Table 9). Moreover, higher air classifier wheel speeds resulted in higher protein content and lower mass yields of the fine fractions from the same raw material (Table 9, the barley endosperm fraction). As expected, with bran materials the median particle size of the milled raw material correlated negatively with the mass yield of the fine fraction in air classification (i.e. the

finer the raw material, the higher the fine fraction mass yield; Table 9). On the contrary, considerably lower fine fraction mass yield was obtained from the barley endosperm fraction (III; the raw material with a median particle size of 18 µm resulted in a mass yield of 6.4% when air classified at 21 500 rpm) when the comparison was made based on the median particle sizes of the raw materials processed with similar air classification parameters (the median particle size of the pin disc-milled rice bran raw material (I) was 62 µm and resulted in mass yield of 27.2% when air classified at 21 000 rpm; Table 9) despite the application of flow aid in barley fractionation.

Air classification of the defatted rice bran was carried out in one- and two-step processes (I). In the one-step approach the aim was to increase protein content directly after pin disc milling. In air classification, a fine fraction with 25.7% protein content was produced with a mass yield of 27.2% from the defatted rice bran and the protein transferred to that fraction corresponded to 38.0% of the raw material protein (Table 9). In addition to protein enrichment, which was also proven by microscopy (Figure 7a vs 7b), a clear increase in the amount of phytic acid to 21.6% took place, the SDF:IDF ratio increased from 0.2 to 0.5 and starch content decreased to 7.9% (Table 9). In accordance with the reduced IDF content of the fine fraction, large intact cell wall components and pericarp structures were absent in the microstructure of the protein-enriched fraction (Figure 7b). One-step air classification processes were also applied in the fractionation of wheat and rye brans (II). Somewhat higher protein contents of 30.9 and 30.7% were achieved in the fine fractions produced from wheat and rye brans, initially containing 16.4 and 14.7% protein, respectively, when compared with rice bran (Table 9). However, the mass yields in wheat and rye bran fractionations remained lower, at 9.6–12.9%, thus resulting in lower PSE values of 18.0–26.9% compared with rice bran. Interestingly, starch content was clearly less affected in the fractionation of wheat and rye brans compared with rice bran. Phytic acid enrichment to the protein-rich fraction was evident for all brans, and increases in the SDF:IDF ratios were noticed to also take place during the fractionation of wheat and rye brans (Table 9).

In the two-step fractionation process of defatted rice bran (I), instead of direct protein enrichment, the first fractionation step targeted the separation of the bran preparation into one fraction free of pericarp structures and another composing of the pericarp and intact aleurone cells enclosing proteins. Indeed, the coarse fraction from the first step contained intact aleurone structures on the basis of microscopy analysis (Figure 7d), which guided the further processing of the fraction by pin disc milling for cell wall disruption. The fine fraction from the first step was free from pericarp structures and composed of broken aleurone cell walls and loose and free protein (Figure 7c). The protein content was not largely affected in the first air classification step (19.7% in the fine fraction vs 18.5% in the raw material), whereas starch content decreased from 23.5 to 12.9% (Table 9). Both the phytic acid content and SDF:IDF ratio increased to higher levels compared with the raw material and the fine fraction

from the one-step air classification. Pin disc milling and further air classification of the coarse fraction from the first fractionation step of the two-step air classification process resulted in protein enrichment up to 27.4% with a total mass yield of 13.9% and total PSE of 20.2% from the raw material. Thus, the highest protein content for rice bran was reached in the two-step air classification process.

Pre-mixing the barley endosperm raw material with a flow aid increased the mass yield of the fine fraction from 5.3 to 6.3%, protein content from 26.3 to 28.3% and PSE from 16.9 to 21.6% during air classification with the highest applied air classifier wheel speed (Table 1 in III). The barley endosperm raw material differed from the bran raw materials as it contained much more starch, less protein and the DF content was notably low. Nevertheless, protein enrichment to similar levels (22.3–28.3%) as for the brans was achieved, allowing concomitantly high PSE values (59.4–21.7%; Table 9). Higher protein content and lower PSE accounted for the fraction produced with the highest air classifier wheel speed, as anticipated. Reduction in starch content to 64.3–55.3% correlated positively with increased protein content. No major changes between the ratios of the DF components were observed during the air classification of the barley sample. A comparison of the microstructures of the barley raw material and protein-enriched fractions revealed the size-based fractionation of starch granules and fibrous cell wall structures (Figure 7e–h) and that the fine fractions contained more small starch granules embedded in a continuous protein matrix (Figure 7f and 7h). The fractionation process that aimed at high PSE from the barley endosperm fraction was scaled up and the protein-enriched fraction obtained in the industrial-scale process (24.0% protein, 20.6% mass yield, 52.9% PSE) was well comparable to the pilot-scale fraction (22.3% protein, 22.1% mass yield, 59.4% PSE).

The partitioning of different protein classes between the air-classified fractions and raw materials was detected on a reducing SDS-PAGE gel when protein band intensities were compared (Figure 8). Evaluation of the protein profiles of the rice bran ingredients revealed the enrichment of the proteins with molecular weights around 18–20, 30–35 and 55 kDa to all of the three fine fractions, whereas the fine fractions contained fewer of the proteins with molecular weights around 10, 16, 22–25, 50 and 53 kDa when compared with the raw material bran. In wheat bran, the proteins at 10, 17–18, just below 25, 32 and 50 kDa were enriched in the fine wheat bran fraction and the amounts of the proteins at around 14, 20 and 25 kDa were reduced in that fraction. For rye bran, the proteins with molecular weights of 12–14, 30, 40, 50, 55 and 100 kDa showed enrichment in the fine fraction. For both wheat and rye brans, aggregates sizing >250 kDa were detected in the raw material brans but were absent in the fine fractions.

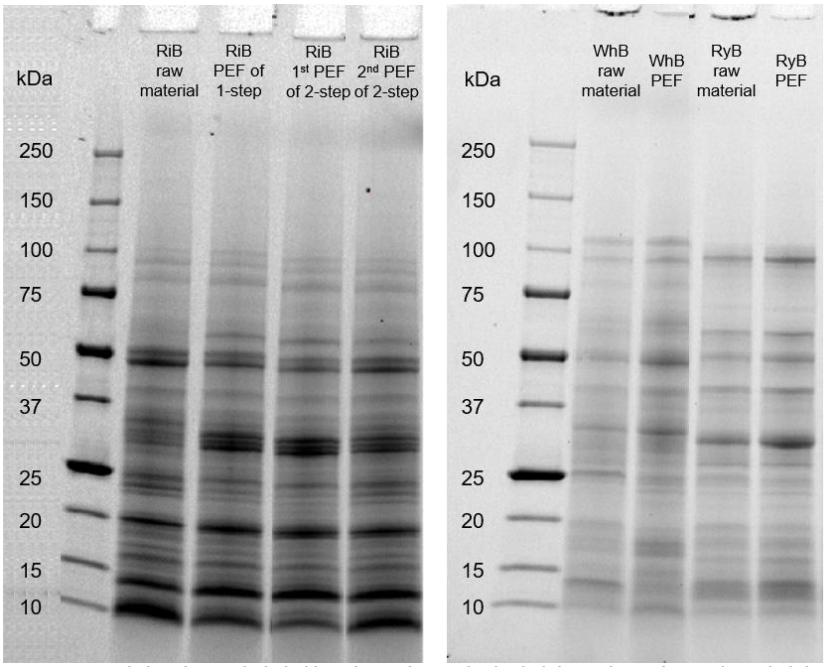

Figure 8. SDS-PAGE of rice bran (RiB) (I), wheat bran (WhB) (II) and rye bran (RyB) (II) raw materials and the protein-enriched fractions (PEFs) produced by air classification when analysed under reducing conditions.

5.2 TECHNO-FUNCTIONAL PROPERTIES OF THE DRY-FRACTIONATED INGREDIENTS

The techno-functional properties (i.e. protein solubility, dispersion stability, pasting properties, foaming properties, emulsification, WBC and OBC) of the pre-processed raw materials and air-classified fractions were analysed in order to predict the impact of dry fractionation on the food applicability of cereal side stream ingredients (Table 10).

5.2.1 PROTEIN SOLUBILITY (I, II, III)

The protein solubility of the pre-processed rice bran (I), wheat bran (II) and rye bran (II) at native pH (6.7–6.8) in water was 48.5, 43.9 and 42.2%, respectively, and for all the brans, the solubility increased significantly at pH 8 and decreased significantly at pH 5 (Table 10). The ultra-fine milling of wheat and rye brans (II) decreased the solubilities at all the studied pH values (Table 10). All the three air-classified fine fractions produced from the defatted pin disc-milled rice bran exhibited higher solubilites than the raw material at the same pH values. On the contrary, the fine fraction produced from the rye bran showed lower solubility than the raw material. In the case of wheat bran, the protein-enriched fraction was more soluble at native pH and pH 8, whereas it showed lower solubility at pH 5 compared with the raw material bran. The protein solubility of the barley endosperm raw material fraction was not analysed in the current study, but for the protein-enriched barley endosperm

fraction (III), a similar trend as for the bran samples regarding the impact of pH on the solubility was observed, that is, the solubility increased from 9.0 to 22.2% when pH was increased from 5 to 8 (Table 10). However, also at more acidic (pH 3) and alkaline (pH 9) conditions, lower solubilities of 19.6 and 28.9%, respectively, were observed for the protein-enriched barley fraction (Figure 5 in III) when compared with the protein-enriched bran fractions. When the barley protein isolate was investigated, higher solubilities of 51.4 and 64.3% were observed at acidic pH 3 and alkaline pH 9, respectively, whereas the solubility at pH 5 was even lower (2.9%) than that of the protein-enriched fraction (Figure 3 in V).

5.2.2 OTHER TECHNO-FUNCTIONAL PROPERTIES (I, II, III)

In all the studied cereal side stream samples deriving from wheat (II), rye (II) and barley (III), the protein-enriched fractions showed lower WBCs and OBCs compared with the milled raw materials. The WBC and OBC values of different protein-enriched fractions were 1.2 and 1.0–1.1%, respectively (Table 10). For wheat, the pin disc-milled bran showed the highest WBC (2.7 g/g) and OBC (1.3 g/g) among the wheat bran samples. For rye, the pin disc-milled bran delivered the highest OBC (1.4 g/g) whereas ultra-fine milling improved the WBC from 1.5 to 1.9 g/g.

The aqueous dispersions of the protein-enriched fractions derived from both rice and wheat brans exhibited good dispersion stabilities, devoid of sedimentation at their native pH during a 30 min observation time (Table 10; Figure 4 in I; Figure 4 in II). On the contrary, the dispersion of the protein-enriched fraction from rye bran already started to sediment at 10 min and was clearly phase separated by 30 min (Figure 4 in II). However, the dispersion stabilities of all the protein-enriched fractions and the ultra-finely milled wheat and rye brans were clearly improved compared with the pin disc-milled raw materials. Ultra-finely milled wheat and rye brans sedimented faster than the corresponding protein-enriched fractions (Figure 4 in II).

The pasting properties of wheat (II), rye (II) and barley (III) raw materials and protein-enriched fractions were evaluated using the RVA in order to observe the behaviour of the ingredients during heating and subsequent cooling. The peak and final viscosity values correlated well with the starch content as the lowest viscosities were observed for the wheat bran ingredients and the highest for the barley endosperm samples, the highest value corresponding to the barley endosperm raw material that contained 80.0% starch (Table 10). For wheat bran samples, the lowest peak and final viscosities were achieved by the protein-enriched fraction, whereas for rye bran ingredients, only the peak viscosity was the lowest for the protein-enriched fraction. For wheat bran, the ultra-fine milling did not induce major differences in viscosities compared with the pin disc-milled raw material, whereas for rye bran, the ultra-fine milling almost doubled both the peak and final viscosity values.

Table 10. The impact of dry fractionation on the techno-functional properties, including protein solubility, and water and oil binding capacities, as well as dispersion stability and pasting properties, of cereal side stream ingredients (I, II, III).

Raw material	Fraction	Protein solubility (%)			WBC (g/g)	OBC (g/g)	Other techno-functional properties (dispersion stability, emulsification, foaming, and pasting properties)	Publi-cation
		pH 5	pH 6.7–6.8	pH 8				
Rice bran, SC-CO_2 extracted	Pin disc-milled 2 x 17 800 rpm	30.0 ± 0.4	48.5 ± 0.2	66.6 ± 0.7	na	na	Poor dispersion stability	I
	Fine from 1-step AC	38.1 ± 0.2	66.9 ± 0.2	82.9 ± 0.6	na	na	Stable dispersion at 30 min	
	Fine from 1st step of 2-step AC	46.2 ± 0.5	74.9 ± 2.2	82.6 ± 0.4	na	na	Stable dispersion at 30 min	
	Fine from 2nd step of 2-step AC	35.7 ± 0.3	58.3 ± 0.6	81.7 ± 0.5	na	na	Stable dispersion at 30 min	
Wheat bran	Dried, 0.3 mm sieve- and pin disc-milled 2 x 17 800 rpm	38.5 ± 0.6	43.9 ± 0.3	65.6 ± 0.5	2.7 ± 0.04	1.3 ± 0.01	Poor dispersion stability; PV: 158 cP; FV: 222 cP	II
	Ultra-finely milled	34.3 ± 0.7	38.2 ± 0.4	53.8 ± 0.8	2.2 ± 0.03	1.1 ± 0.02	Limited dispersion stability at 10 min; no change in emulsion PSD in 1 d but minor clarification on the top; PV: 146 cP; FV: 280 cP	
	Fine fraction from 1-step AC	30.1 ± 1.0	45.1 ± 0.5	75.5 ± 0.5	1.2 ± 0.04	1.0 ± 0.01	Stable dispersion at 30 min; no change in emulsion PSD in 1 d but minor clarification on the top; PV: 92 cP; FV: 167 cP	
Rye bran	Dried and pin disc-milled 2 x 17 800 rpm	38.4 ± 1.2	42.2 ± 0.5	50.5 ± 0.2	1.5 ± 0.01	1.4 ± 0.00	Poor dispersion stability; PV: 496 cP; FV: 580 cP	
	Ultra-finely milled	36.6 ± 0.4	40.2 ± 1.0	47.2 ± 1.0	1.9 ± 0.01	1.1 ± 0.01	Limited dispersion stability at 10 min; no change in emulsion PSD in 1 d but minor clarification on the top; PV: 789 cP; FV: 1032 cP	
	Fine fraction from 1-step AC	31.9 ± 1.2	34.9 ± 0.6	43.6 ± 0.1	1.2 ± 0.07	1.0 ± 0.02	Limited dispersion stability at 10 min; no change in emulsion PSD in 1 d but minor clarification on the top; PV: 427 cP; FV: 598 cP	
Barley endo-sperm fraction	Raw material	na	na	na	na	na	PV: 5870 cP; FV: 6118 cP	III
	Fine fraction from 1-step AC at 8 000 rpm	9.0 ± 0.1	16.6 ± 0.2	22.2 ± 0.2	1.2 ± 0.01	1.1 ± 0.02	Limited foaming capacity and stability observed at all the studied pH-values (3, 5.1, 7, 8); PV: 3113 cP; FV: 3634 cP	

WBC: water binding capacity; OBC, oil binding capacity; AC: air classification; na: not analysed; PV: peak viscosity in a Rapid Visco Analyser (RVA); FV: final viscosity in the RVA; PSD: particle size distribution.

The emulsification ability of wheat and rye bran ingredients was studied by homogenising the aqueous sample dispersions with oil using an ultrasound-assisted method (II). Both the protein-enriched fractions and the ultra-finely milled bran raw materials resulted in stable emulsions that showed only minor phase separation during one-day storage and the emulsions did not coarsen remarkably based on particle size analysis during the observation period (Table 10; Figures 5–6 in II). The foaming capacity and stability of the protein-enriched barley fraction (III) were assessed and, at the studied pH values of 3, 5.1, 7 and 8, showed low values of 48–90% and 0–48%, respectively (Table 3 in III). The instability of the foams was also detected as high drainage (87–92%) of the liquid fraction from the foam already 10 min after foaming.

5.3 FUNCTIONALISATION OF THE AIR-CLASSIFIED INGREDIENTS

5.3.1 PHYTASE TREATMENT (IV)

The potential impact of phytic acid degradation using phytase on the techno-functional properties of the protein-enriched rice bran fraction was elucidated in Publication IV. Phytase treatment lowered the phytic acid content of the protein-enriched rice bran fraction to 1–3%, depending on the solid content of the treatment. The phytase-treated sample exhibited higher protein solubility in water at acidic pH than the control sample (Table 11). The most pronounced impact was seen at pH 2 where the solubility of the control sample was 42%, whereas it reached 55% for the phytase-treated sample. No major differences in the solubilities were observed at neutral and alkaline pH. On the other hand, surface hydrophobicity at pH 6.7 was increased from 36 to 87 as a result of the phytase treatment (Table 11). The heat treatment of the control and the phytase-treated dispersions led to a dramatic decrease in surface hydrophobicity to values of 27 and 20, respectively.

The applicability of the protein-enriched rice bran fraction with and without a pre-phytase treatment in a heat-induced gel system was investigated at pH-values 5, 6.7 and 8. At the end of the heating and cooling cycles applied in a rheometer, the final G' values of the control and phytase-treated gels prepared at pH 5 were 90 and 86 Pa, respectively, whereas the gels prepared at pH 6.7 exhibited values of 1057 and 1065 Pa, respectively (Table 11). Likewise, the final loss tangent (tan δ) (0.19), yield strain (0.33 and 0.25) and WHC (35.9% and 38.0%) of the gels did not considerably differ between the control and the phytase-treated samples at pH 5. At pH 6.7, tan δ decreased to 0.18 and 0.15 and WHC increased to 53.5 and 62.2% for the control and the phytase-treated samples, respectively. The yield strain of the control sample at pH 6.7 remained at 0.33%, similarly to that observed for the control sample at pH 5, whereas the phytase-treated sample at pH 6.7 showed the higher value of 0.65. However, the results show that no major differences were detected as a

result of phytase treatment under these pH conditions, and stronger gels were formed at neutral pH than at slightly acidic pH. At slightly alkaline pH 8, on the other hand, considerable differences were observed between the phytase-treated and control samples. The control sample did not differ greatly from the properties of the samples gelled by heating at pH 6.7, whereas the phytase-treated sample exhibited a tenfold higher G' compared with the control. Moreover, the phytase-treated sample at pH 8 showed the highest WHC and the lowest final tan δ among all the samples. On the contrary, this sample also exhibited a low yield strain of 0.26%. Differences in the microstructures of the gels (Figure 5 in IV) were minor and only a slightly more uniform and homogenous distribution of proteins and cell wall glucans was observed in the phytase-treated gel at pH 8 compared to the other gels.

Table 11. The impact of phytase treatment on the protein solubility, surface hydrophobicity and heat-induced gelation of the protein-enriched rice bran fraction (IV).

pH	Sample	Protein solubility (%)[a]	Surface hydro-phobicity (before / after heating)[a]	Gel properties			
				Final G' (Pa)[b]	Final Tan δ[b]	Yield strain (%)[b]	WHC (%)[a]
2	Control	42 ± 2	na	na	na	na	na
	Phytase	55 ± 0	na	na	na	na	na
4	Control	41 ± 1	na	na	na	na	na
	Phytase	47 ± 0	na	na	na	na	na
5	Control	na	na	90 ± 6	0.19 ± 0.00	0.33 ± 0.07	35.9 ± 2.6
	Phytase	na	na	86 ± 3	0.19 ± 0.01	0.25 ± 0.00	38.0 ± 1.8
6	Control	46 ± 0	na	na	na	na	na
	Phytase	45 ± 0	na	na	na	na	na
6.7	Control	46 ± 0	40 ± 6 / 27 ± 1	1057 ± 83	0.18 ± 0.00	0.33 ± 0.07	53.5 ± 1.9
	Phytase	45 ± 1	87 ± 10 / 21 ± 2	1065 ± 47	0.15 ± 0.00	0.65 ± 0.00	62.2 ± 4.3
8	Control	49 ± 0	na	1141 ± 16	0.13 ± 0.01	0.26 ± 0.01	54.6 ± 0.6
	Phytase	46 ± 0	na	11336 ± 564	0.10 ± 0.00	0.26 ± 0.00	77.8 ± 0.3
10	Control	52 ± 0	na	na	na	na	na
	Phytase	50 ± 1	na	na	na	na	na

[a] ± standard deviation; [b] ± average deviation; WHC: water holding capacity; na: not analysed

5.3.2 ULTRASOUND TREATMENT (V)

Production of the protein-enriched barley fraction with high PSE was repeated at an industrial scale and the impact of ultrasound treatment and pH shifting on physicochemical ingredient properties was investigated (V). In addition, a protein isolate was prepared from the protein-enriched fraction using alkaline extraction and isoelectric precipitation. The differences between the two barley

protein ingredients (i.e. the protein-enriched fraction from the air classification, 24.0% protein, 55.6% starch; Table 9; Table 1 in V, and the protein isolate 85.9% protein, 0.6% starch; Table 1 in V), affected by ultrasound treatment and pH shifting, alone or in combination, were studied. The protein-enriched barley fraction and barley protein isolate exhibited solubilities of 14.7 and 2.9% at their native pH conditions of 5.9 and 5.0, respectively (data not shown). At pH 3, higher solubilities of 18.8% and 51.4% were detected for the protein fraction and isolate respectively, at pH 7 the corresponding percentages were 22.4% and 16.1%, and at pH 9 they were 37.3% and 64.3% (see the control samples in Table 12). Adjusting the pH to 9 improved the colloidal stability of both the protein-enriched fraction and the isolate, although the effect was more pronounced for the latter (Figure 5 in V). For both materials, colloidal stabilities were rather poor and similar at pH 3 and 7.

Table 12. The impact of ultrasound treatment and pH shifting on the protein solubility, particle size and colloidal stability of the protein-enriched barley fraction and barley protein isolate (V, particle size median values are unpublished data).

Sample	Treatment pH	Sample	Analysis pH	Protein solubility (%)[a]	Median particle size (µm)[b]	Colloidal stability at 3 h when compared to the same control sample at pH 7
Protein-enriched barley fraction	3	Control	3	18.8 ± 1.1	23.3 ± 3.6	No changes
			7	15.3 ± 1.7	25.7 ± 3.9	No changes
		Ultrasound	3	18.0 ± 0.8	4.5 ± 0.1	Slightly improved
			7	15.7 ± 1.7	5.5 ± 0.3	Slightly improved
	7	Control	7	22.4 ± 1.2	27.5 ± 2.5	-
		Ultrasound	7	24.2 ± 1.1	4.9 ± 0.2	Clearly improved
	9	Control	9	37.3 ± 1.1	18.6 ± 1.7	Slightly improved
			7	26.1 ± 1.8	19.9 ± 0.9	Slightly improved
		Ultrasound	9	60.3 ± 3.1	4.3 ± 0.1	Clearly improved
			7	40.7 ± 2.8	4.2 ± 0.1	Clearly improved
Barley protein isolate	3	Control	3	51.4 ± 5.1	na	No changes
			7	11.2 ± 2.3	na	No changes
		Ultrasound	3	69.7 ± 10.1	na	Clearly improved
			7	11.2 ± 4.3	na	Clarification on top
	7	Control	7	16.1 ± 1.0	51.7 ± 5.2	-
		Ultrasound	7	25.6 ± 1.0	1.4 ± 0.1	Clearly improved
	9	Control	9	64.3 ± 5.5	na	Clearly improved
			7	38.3 ± 1.5	na	Clearly improved
		Ultrasound	9	74.3 ± 4.3	na	Clearly improved
			7	46.0 ± 6.7	na	Clearly improved

[a] ± standard deviation; [b] ± average deviation; na: not analysed

Shifting the pH first to pH 3 and then back to neutral pH 7 did not improve the protein solubility or colloidal stability of either of the fractions compared to their solubilities or stabilities measured directly at pH 7 (Table 12). On the contrary,

shifting to pH 9 and back to pH 7 induced slight improvements in solubility and colloidal stability of the protein-enriched fraction. Interestingly, the similar shifting applied for the isolate improved both solubility (showing an increase from 16.1 to 38.3%) and colloidal stability considerably, when compared with the sample only adjusted to pH 7.

The effect of ultrasound treatment was assessed by comparing the properties of the samples at different pH values with and without ultrasonication. In regard to the protein-enriched fraction, a clear impact of ultrasound treatment was only noticed at pH 9, where sonication increased the solubility from 37.3 to 60.3% (Table 12). Nevertheless, the colloidal stability was clearly improved at both pH 7 and pH 9 (Figure 5 in V). For the isolate, ultrasound treatment improved protein solubility at all the studied pH conditions, as well as colloidal stability at pH 3 and 7, whereas at pH 9, the sample was stable both with and without the sonication (Table 12). For both samples ultrasonication decreased the particle size considerably. For the protein-enriched fraction, the median particle sizes were reduced from 18.6–27.5 to 4.2–5.5 μm in ultrasound treatment. For the isolate, a more intensive reduction from 51.7 to 1.4 μm at pH 7 was observed during sonication. Differences between the protein profiles of the protein-enriched fraction and the isolate were minor based on SDS-PAGE analysis. The ultrasound treatment did not modify the clearly observed bands in the gels, although more smearing was noticed in the lanes of the sonicated samples (Figure 2 in V).

Ultrasound treatment at pH 3 or pH 9, followed by pH shifting to neutral pH 7, improved both colloidal stability and protein solubility in selected samples. The protein-enriched fraction had the most notable change resulting from ultrasonication at pH 9 and pH shifting to 7, where the colloidal stability was clearly improved (Figure 5 in V) and solubility reached 40.7% (Table 12). Protein solubility remained at 26.1% after pH shifting to 9 and back to 7 (without ultrasonication) and at 24.2%, after ultrasonication directly at pH 7 (Table 12). Solubility was the highest for the ultrasonicated sample at pH 9 (60.3%). Likewise, shifting the pH of the ultrasonicated protein isolate from pH 9 to 7 resulted in higher protein solubility (46.0%) compared with the sample ultrasonicated at pH 7 (25.6%). The impact on protein solubility was only slightly improved when compared to the control sample, shifted to pH 9 and back to pH 7 (38.3%) without the ultrasonication step. On the contrary, pH shifting to acidic conditions prior to ultrasound treatment and adjustment to neutral had no significant impact on the protein solubility of either of the barley protein ingredients.

6 DISCUSSION

The work elucidated dry fractionation of cereal side streams including rice bran (I), wheat bran (II), rye bran (II) and a barley endosperm fraction (III), aiming at enrichment of proteins and other nutritionally and functionally relevant components, such as DF. Before the dry fractionations the raw materials were pre-treated with the different approaches selected based on the initial raw material properties. Considerable differences between the raw materials in terms of their behaviour in fractionation were observed. Moreover, techno-functional properties differed both between fractions and raw materials. Functionalisation approaches targeted at improving ingredient applicability in high-moisture food systems, namely phytase treatment for the protein-enriched rice bran fraction (IV) and ultrasound treatment with or without pH shifting for barley protein ingredients (V), were effective. In this section, the factors affecting the observed phenomena are discussed.

6.1 EVALUATION OF DIFFERENCES IN DRY FRACTIONATION OF CEREAL SIDE STREAMS

6.1.1 THE EFFECT OF PRE-TREATMENTS ON INGREDIENT PROPERTIES AND DRY FRACTIONATION EFFICIENCY

Analysis of the biochemical composition of the cereal raw materials prior to dry processing guided selection of the most potential pre-prosessing techniques that were hypothesised to aid in dry fractionation. The applied pre-treatments included defatting with SC-CO_2 for rice bran (I), drying for wheat and rye brans (II) and mixing with a flow aid (III) for the barley endosperm fraction (Table 13).

The high fat content of rice bran is known to induce rancidity unless removed or stabilised and in this work it was also expected to disable dry milling of the bran, and therefore, fat extraction was included as a pre-processing step for rice bran. Indeed, pin disc milling of a full-fat bran in the pre-experiments of the current study was proven inapplicable whereas efficient particle size reduction and further component fractionation was achieved in pin disc milling and air classification of the defatted bran, respectively. In compliance with these findings, Sibakov et al. (2011), Xing et al. (2018) and Flynn et al. (2019) have reported that the high fat content of the raw materials affect the dry processes negatively. Traditionally, fat extraction has been carried out using solvents, such as hexane. However, solvents are regarded hazardous and traces of them are considered inappropriate and unsafe in human foods. On the contrary, SC-CO_2 is a more gentle fat-removal agent used for rice bran oil extraction that applies relatively low extraction

temperatures (40–90°C) and high pressures (200–500 bar), transforming the carbon dioxide into a supercritical state that diffuses into the raw material matrix and removes the extractables while diffusing out from the matrix as a result of lowering the temperature or pressure (Kuk and Dowd 1998; McHugh and Krukonis 2013). Furthermore, carbon dioxide is non-toxic and non-flammable, and the exploitation of only low temperatures in extraction makes it suitable for heat-sensitive materials (Patel 2005). In this work, the selection of the defatting method was done based on the gentle nature of SC-CO_2 extraction, which retains the native functional properties of the rice bran proteins. This was proven by the high protein solubility of the defatted bran (30–67% between pH 5 and 8) and by the microstructures of the defatted bran samples, which were devoid of any protein aggregates and revealed unstained areas inside the aleurone cells, suggesting the removal of oil bodies from inside the aleurone cells in defatting. On the contrary, most of the previous studies on the dry fractionation of rice bran for protein enrichment have included solvent-extraction or parboiling steps which may have led to protein denaturation or component complexation due to heating or drying, and thus makes direct comparison to the prior literature challenging (Jayadeep et al. 2009; Saio and Noguchi 1983; Tang et al. 2002).

One potential factor that limits bran protein enrichment via dry processing is associated with the rigid structures of bran, more specifically, the aleurone cell walls enclosing the proteins (Arte et al. 2016; Hemery et al. 2011; Nordlund et al. 2013b; Rosa-Sibakov et al. 2015a). The microstructural observations in the present work revealed that in the defatted rice bran, the aleurone layer cells were partly broken and some intracellular material seemed to have leaked out from the cells of the raw material, most probably due to the rice bran production process relying on pearling and polishing, which may already result in structural disintegration of the bran layers. Thus, efficient particle size reduction was obtained for rice bran by only pin disc milling. On the contrary, the well-known hardness of the aleurone cell structures of wheat and rye bran (Hemery et al. 2011; Rosa-Sibakov et al. 2015b) directed studying treatments that targeted improved cell wall degradation prior to the dry fractionation of these brans and aimed at protein liberation and further protein enrichment from inside the aleurone cells. In the present work, drying the materials prior to milling was applied in order to increase their brittleness, something which has been reported to take place in drying (Laskowski and Lysiak 1999; Tyler and Panchuk 1982), and aid in particle size reduction, which would potentially improve the release of the proteinaceous structures from inside the cell walls. In line with the previous studies, particle size was reduced more efficiently whereas protein content in the air-classified protein-enriched fractions was not largely affected by the pre-drying, resulting in slight increases in the PSEs.

Table 13. The impact of pre-treatments on improving the behaviour of the cereal raw materials in dry fractionation.

Raw material	Pre-treatment	Justification for the need for pre-treatment	Proposed impact of pre-treatment	Publication
Rice bran	Defatting with SC-CO_2	Milling of high-fat materials is known to be challenging and component separation is improved in defatted cereal materials (Flynn et al. 2019; Sibakov et al. 2011; Xing et al. 2018).	Defatting enabled milling, which was not possible to be performed for the full-fat bran. Based on microscopy, no protein aggregation took place during SC-CO_2 extraction and thus protein enrichment was possible.	I
Wheat bran and rye bran	Drying for 48 h at 40°C	Bran materials, especially wheat bran, are known to be relatively resistant to particle size reduction by dry processing. The cell walls physically limit protein separation from protein-rich aleurone cells (Hemery et al. 2011; Rosa-Sibakov et al. 2015a).	Drying increased the brittleness of the material, which allowed improved particle size reduction and subsequently higher mass yield of the protein-enriched fraction. This was postulated to mostly result from the breakage of the cell walls liberating protein, and therefore, the protein enrichment level was not compromised.	II
Barley endo-sperm fraction	Mixing with 0.5% Aerosil 200F flow aid	High-starch materials with relatively fine particle size tend to flow poorly inside dry processing equipment (Dijkink et al. 2007).	Mixing the material with a flow aid improved flowability by decreasing cohesion and van der Waals forces between the particles, which further aided protein separation and resulted in higher mass yield of the protein-enriched fraction without compromising the protein content reached.	III

In regard to particle size reduction, the present work also investigated the ultra-fine milling of wheat and rye brans (II). Ultra-fine milling was studied to allow better comparison of the protein-enriched fractions produced by air classification and having small particle sizes with the non-fractionated brans since particle size is recognised to have a considerable impact on technological ingredient functionality. Therefore, a comparison of the fine fractions with the ultra-finely milled brans rather than the coarser pin disc-milled brans was considered important. In ultra-fine milling, the pre-drying step was omitted, firstly, since its effect was supposed to be minor when the more impactful milling was applied and, secondly, so as not to further increase the energy consumption of the intensive milling process. However, continuing the dry processing of the ultra-finely milled brans by air classification was not considered to have potential for various reasons. First, reducing the particle size of the non-protein components in ultra-fine milling would restrict protein

enrichment in the following air classification step. Second, damaged starch content increases in extensive milling (Berton et al. 2002; Drakos et al. 2017a; Niu et al. 2014; Tester 1997), again limiting the protein fractionation in air classification due to formation of small-sized starch fragments (Létang et al. 2002; Lundgren 2011; Pelgrom et al. 2013). Third, too fine milling lowers the flowability of the flour particles (Pelgrom et al. 2013). Finally, the potential heat generation during milling, resulting in protein denaturation or particle aggregation, which have earlier been reported to take place in intensive milling (Drakos et al. 2017a; Rommi et al. 2015b; Van Craeyveld et al. 2009), was suggested to limit protein separation by dry means. Indeed, particle aggregation or protein denaturation may be supported by the observed lowered protein solubility of the ultra-finely milled brans compared with only pin disc-milled brans, as discussed in more detail in Section 6.2.1.

Regarding the barley endosperm fraction, which can be considered a side stream fraction from the dry separation of barley β-glucan, the main limitation in dry processing was associated with the small particle size and high starch content. Small protein particles may be attached to the larger starch granules as it is known that particles <200 µm are cohesive (Teunou et al. 1999) and small particles tend to adhere to the surfaces of larger particles (Möller et al. 2021). Dijkink et al. (2007) showed that high starch content decreased powder dispersability in air, which is an essential property in air classification. Flowability can be improved by the addition of Aerosil flow aid to flour, which enlargens the particle–particle distance and reduces van der Waals forces (Müller et al. 2008; Pelgrom et al. 2014). During air classification, both the mass yield and PSE of a protein-enriched lupine fraction were more than doubled by using flow aids despite 10% less protein content being reached (Pelgrom et al. 2014). In the current work, more moderate increases in mass yield and PSE were observed (an increase from 5.3 to 6.3% and an increase from 16.9 to 21.6%, respectively). In contrast to the results of Pelgrom et al. (2014), the protein content of the fine fraction was also increased in this study (from 26.3 to 28.3%) when the flow aid was used, implying that the addition of the flow aid in the barley endosperm fraction improved more efficiently the flow properties. Furthermore, assuming that all the added Aerosil ended up in the fine fraction, the actual PSE was even higher than reported.

6.1.2 PROTEIN ENRICHMENT FROM CEREAL SIDE STREAMS IN RELATION TO THEIR STRUCTURE AND COMPOSITION

Protein enrichment from plant raw materials is traditionally carried out by wet extraction. However, dry fractionation can offer a sustainable and energy efficient alternative to wet processing although the dry processing of cereal grains only results in a moderate enrichment of the desired components. Understanding the cellular architecture of the grain is critical in order to enhance the dry fractionation efficacy of the different components. During protein enrichment from cereal raw materials, the relationship between the

morphology and size of starch and protein constituents plays a crucial role. The similarities between the sizes of protein bodies (0.5–5 µm) (Juliano and Bechtel 1985; Pernollet 1978) and B-type small starch granules in wheat, rye and barley subaleurone and aleurone regions (Goering et al. 1973; Heneen and Brismar 1987; Takeda et al. 1999) limit protein fractionation. In this work, a rather even distribution of starch in the fine and coarse fractions was observed during the air classification of wheat and rye brans as the starch content of the fine fraction did not considerably differ from that of the raw material. This supports both the presence of small-sized starch granules in the subaleurone region of the starchy endosperm of wheat and rye, and their fractionation to the fine fraction, together with the protein, while the larger granules separate to the coarse fraction. It is noteworthy that cereal brans botanically contain no starch and considerable variations in the starch contents of brans from different species result from the differing amounts of starchy endosperm present in the bran preparations. As the starch contents did not increase in the air classification of wheat and rye brans in this work, the presence of low amounts (2.0–2.2%) of damaged starch in the pin disc-milled wheat and rye bran raw materials was not presumed to have affected the protein fractionation. Despite the even distribution of starch granules in the air classification of dried and pin disc-milled wheat and rye brans, the protein contents were increased in air classification from 16.4 and 14.7%, respectively, to 30.9 and 30.7%, respectively, which are higher values than previously reported in literature for wheat bran air classifications (Ranhotra et al. 1994).

In mature starchy endosperm, such as in the barley endosperm fraction, the starch granules are embedded in a matrix formed of storage proteins (Darlington et al. 2000). As the proteins in the endosperm are not located in separate structural units like protein bodies, the enrichment of proteins applying size- and density-based approaches relies rather on the distribution of starch granules and protein clusters in small and large particles in the milled flour. In this work, the small-sized starch granules of the barley endosperm fraction were found to contain a denser protein matrix around them, whereas around the large granules, the protein matrix was less dense. Thus, protein enrichment was obtained by enriching the small particles where a dense protein matrix was surrounding the small starch granules. However, further enrichment was considered to not be plausible due to the presence of small starch granules in all barley raw materials, and this was also proven when finding the balance between high protein content and low mass yield in this work.

In rice, individual starch granules (3–9 µm) are located inside amyloplasts (7–39 µm) (Juliano 1985; Saio and Noguchi 1983). The one-step air classification of defatted and pin disc-milled rice bran allowed decreasing the starch content of the fine fraction from the original 23.5 to 7.9%, suggesting that the applied pre-processing steps (i.e. SC-CO_2 extraction and pin disc milling) did not degrade the amyloplasts into separate starch granules and

enabled starch fractionation into the coarse fraction. On the contrary, single starch granules are shown to be released from the compound granules (i.e. the amyloplasts) during the wetting of rice flours (Saio and Noguchi 1983) and the same may have taken place in other research reporting rice bran air classification in literature applying solvent extraction for bran deoiling, which would partly explain the more limited protein enrichment (from 15 to 16%) reported earlier for rice bran (Saio and Noguchi 1983). In this work, the rice bran protein content was increased from 18.5 to 25.7–27.4%. Another factor improving protein enrichment specifically from rice bran was the potential positive impact of SC-CO_2 extraction, which was proven to not induce protein denaturation and allowed utilising rice bran without a heat-stabilisation step. However, the PSE values in all bran fractionations remained relatively low at 18–38%, evidencing that most of the bran proteins remained in the coarse fraction(s) after air classification. This indicates that the liberation of protein from inside the larger cellular structures was not complete. A similar observation has been previously reported by Antoine et al. (2004b) who demonstrated that 47% of the wheat bran aleurone cells remained at particles >200 µm after pin disc milling. Another reason for the modest protein recoveries can be ascribed to the potential impact of particle size reduction on inducing the adhesion of small-sized particles onto the surfaces of larger particles via the charging of the particles (Möller et al. 2021). Moreover, too efficient particle size reduction of the non-proteinaceous bran components may have diluted the protein content of the fine fractions.

During protein enrichment, a concurrent decrease in the amount of IDF was observed for all the bran raw materials. This was postulated to result from the removal of pericarp structures that are known to be rich in insoluble cellulose and lignin. Indeed, the removal of the pericarp was detected in microscopy analysis of the protein-enriched rice bran fractions. Due to the fact that the incorporation of IDF into food products is considered challenging owing to taste, mouthfeel and technological aspects, the altered ratio between SDF and IDF in the produced hybrid ingredient fractions proposes elevated suitability of these ingredients, especially in high-moisture food applications. In spite of the lowered amounts of IDF, the total amount of DF remained at good levels, allowing gaining the nutritional benefits of DF consumption. An increase in the SDF:IDF ratio in a protein-enriched air-classified wheat flour fraction has also been reported earlier by Létang et al. (2002).

Defining the histological origin of the proteins present in the protein-enriched bran fractions was achieved by studying the phytic acid fractionation in air classification as well as by analysing the protein solubility (Section 6.2.1) and protein profiles. Remarkable enrichment of phytic acid in all of the protein-enriched bran fractions occurred in the present study. Aleurone grains (i.e. the protein bodies of rice, wheat and rye brans) are reported to be rich in phytic acid, and thus, its enrichment provides evidence of the enrichment of aleurone-derived protein (Antoine et al. 2004b; Bohn et al. 2007; Parker 1981; Tanaka et al. 1978; Wada and Lott 1997). Previously, Ranhotra et al. (1994)

showed a similar enrichment of phytic acid into a protein fraction produced from wheat bran by air classification. Antoine et al. (2004b) also reported that pin disc milling, sieving and air classification of the finest fraction from wheat bran sieving resulted in a phytate-enriched fraction (14.3% vs 7.3% in the bran) with a 10% mass yield that was discussed to be mainly (66%) composed of aleurone cell contents and was free of pericarp structures. In this work, SDS-PAGE analysis proved further evidence of the enrichment of aleurone proteins in the protein-enriched fractions. For wheat, proteins at 10, 17–18, just below 25 and 32 kDa are associated with albumin/globulin and were enriched in the protein fraction, whereas some potential small-sized proteins that also account for these protein classes were rather removed from the fraction (De Brier et al. 2015; Schalk et al. 2017). A similar observation was also made in regard to rye bran, where most of the small-sized proteins showed enrichment in the fine fraction. However, some of those proteins may also originate from the secalins of the endosperm (Gellrich et al. 2003; Redant et al. 2017; Schalk et al. 2017). For rice, globulin and some albumin proteins (Amagliani et al. 2017) were hypothesised to be enriched in the protein fractions based on SDS-PAGE. In the case of rice bran, aleurone protein enrichment was further supported by a reduction in the starch content, which proves that endosperm-derived starch granules, potentially enclosed by an endosperm protein matrix, did not enrich in the protein fraction. This also suggests that the protein enrichment was not due to starchy endosperm storage protein enrichment but, rather, due to fractionation of the released protein bodies from inside the aleurone cells. The successful enrichment of protein bodies from rice bran may also partially explain the low protein content reached due to the fact that the protein content of rice aleurone grains is reported to only reach 14% whereas 56% of the grains are composed of phytic acid (Tanaka et al. 1973). In comparison, the protein content of wheat bran protein bodies is 46% and phytic acid content is 40% (Bohn et al. 2007).

For rice bran, in addition to a one-step air classification process, a two-step approach was also investigated. In this process, the first fine fraction was not highly enriched in protein, contained small-sized aleurone cell wall structures and some starch, and it was rather devoid of pericarp structures. Interestingly, further processing of the coarse fraction from the first fractionation step by pin disc milling and air classification allowed protein enrichment up to 27.4%. In addition, the second fine fraction of this process also recovered almost all of the rest of the raw material SDF that was not recovered into the first fine fraction of this process. Hence, the developed two-step fractionation process for rice bran valorisation is considered valuable for recovering the SDF structures. In general, one plausible option for increasing the protein or fibre enrichment level in dry processing is to apply multiple fractionation steps. However, a drawback of multi-step dry processing is that the enrichment takes place at the expense of mass yield. This means that both mass yield and PSE are lowered and, therefore, the feasibility of such multi-step processes, in comparison with one-step processes, should be carefully evaluated. On the

other hand, a light techno-economic assessment of a one-step process concept closely similar to the current dry processing approach applied in wheat bran protein enrichment has already been proven to be promising in terms of concept feasibility (Hytönen and Sorsamäki 2018).

6.2 THE EFFECT OF DRY FRACTIONATION ON THE TECHNO-FUNCTIONAL PROPERTIES OF CEREAL SIDE STREAM INGREDIENTS

Most foods are composed of a multitude of compounds, all of them affecting the product properties. Therefore, the holistic functionality of multi-component protein-enriched hybrid ingredients should be understood. It must be noted that, for the hybrid ingredient, the protein functionality should not be overestimated as the other major components (mainly starch and DF, but also some minor components, such as phytic acid) may have a crucial impact on the overall ingredient applicability. Applying specific techno-functionality measurements can, however, be used as a preliminary tool to estimate the food applicability of the multicomponent systems, and thus, both protein- and carbohydrate-associated analytics were carried out in the current work to explore the hybrid ingredient fractions.

6.2.1 CHANGES IN PROTEIN SOLUBILITY

Protein solubility is the key techno-functional feature that affects certain structure-forming properties of proteins. The solubility of plant proteins depends largely on pH and other environmental conditions. In the current study, the lowest protein solubilities were detected for all the raw materials and protein-enriched fractions in the pH values around the range of reported isoelectric points that are: 4–6.2 for wheat (Arte et al. 2019; Balandrán-Quintana et al. 2015; Csonka et al. 1926; Idris et al. 2003), 4.6–7.6 for rye (Csonka et al. 1926; Meuser et al. 2001) and 5–6 for barley (Bilgi and Çelik 2004; Wang et al. 2010; Yalçin et al. 2008). For rice, various isoelectric points ranging from 4.1 to even 7.9 have been reported (Adebiyi et al. 2007; Amagliani et al. 2017; Chandi and Sogi 2007; Ju et al. 2001).

The impact of protein fractionation on protein solubility at pH 5, 6.7–6.8 and 8 was investigated for rice bran (I), wheat bran (II) and rye bran (II). Interestingly, the observed effects were species specific. The fine fractions of rice bran exhibited higher protein solubilities than the pin disc-milled raw material bran at all three pH values whereas the fine wheat bran fraction was more soluble than the pin disc-milled raw material at neutral and alkaline pH and less soluble at pH 5. On the contrary, the fine rye bran fraction was less soluble than its pin disc-milled raw material at all three pH values. The improved solubilities of the rice and wheat bran fractions in comparison with their raw materials may be explained by the successful enrichment of more

soluble albumin and globulin protein classes from inside the aleurone cells, where they are concentrated in the wheat, rice and rye kernels (Amagliani et al. 2017; Bushuk 2001; De Brier et al. 2015). In particular, the aleurone protein bodies that are rich in phytate are reported to exhibit high protein solubility of 70% in rice (Tanaka et al. 1973). Moreover, the finer particle sizes of the fractions may improve the solubilisation from the protein-enriched fractions as well. De Brier et al. (2015) also reported a considerable increase in the albumin and globulin protein solubilities of wheat bran as a result of ball milling. The lowered protein solubility of the air-classified rye bran fraction in comparison with the raw material remains somewhat challenging to explain. It is possible that the protein liberated from inside the aleurone cells in milling and further fractionated into the fine fraction is partly insoluble by nature. Another possible explanation is the formation of protein aggregates during the pin disc milling of rye bran and the enrichment of those insoluble particles in the protein fraction. Moreover, based on the percentual increments in the phytic acid content of the fine fractions, which reached 90% for rye while being 148–182% and 189% in the one-step air classifications of rice and wheat brans respectively, it may be possible that the enrichment of aleurone-derived proteins was less evident for rye than for other raw materials. Therefore, the enrichment of proteins other than aleurone proteins, such as insoluble starchy endosperm proteins, may have lowered the overall protein solubility in rye. On the contrary, the enrichment of starchy endosperm proteins in the protein-enriched wheat bran fraction was not suggested to have taken place since wheat gluten exhibits low protein solubility at the studied pH values, especially at alkaline pH, and thus should have lowered the protein solubility if enriched (Deng et al. 2016).

The bran protein solubility values were somewhat higher than observed in literature earlier for similar raw materials. For differently milled wheat brans, protein solubility values ranging from 12 to 14% at pH 5.5 and from 14 to 40% at pH 6.5–7.5 have been reported (De Brier et al. 2015; Idris et al. 2003), whereas in this study the values ranged from 30 to 76% between pH 5 and 8. Likewise, for rice, the literature values have ranged from approximately 5 to 15% at pH 5 and from 50 to 60% at pH 8 (Bera and Mukherjee 1989; Gnanasambandam and Hettiarachchy 1995; Zhu et al. 2017), while this study showed solubilities of 30–46% and 67–83% at pH 5 and pH 8 respectively. Limited data is available on rye bran protein solubility in literature. However, solubility of 31.9% in a saline buffer at pH 6.9, reported by Nordlund et al. (2013b), is close to the value 42.2% obtained at pH 6.7 in the current work. The reasoning for the higher solubilities detected in the current work may lie in various factors. First, the ratio between soluble and insoluble proteins in the commercial bran preparations may vary due to differing amounts of insoluble starchy endosperm proteins present in the bran. Second, the differences in the protein extraction methods applied in different studies (i.e. dry fractionation or concentration/isolation via alkaline wet extraction) affecting both protein composition and the degree of protein denaturation, as well as the details of

the protein solubility analysis, all have an impact on the values obtained. Third, as already discussed in the previous chapter, the particle size reduction of the raw material has a prominent impact on the protein solubilisation due to, for example, the release of the proteins physically entrapped by the intact fibrous cell wall structures (Antoine et al. 2004a; Van Craeyveld et al. 2009). However, high-impact milling for particle size reduction may also result in protein aggregation (Drakos et al. 2017b, 2017a; Van Craeyveld et al. 2009). Indeed, the ultra-fine milling applied in the current research for wheat and rye brans lowered the protein solubilities throughout the pH range when compared with the more gently pin disc-milled raw materials, and thus, ultra-fine milling is not considered a suitable processing method for bran materials that are targeted towards applications demanding high protein solubility. The reasoning for the lowered protein solubility was assumed to be the heat generated during milling causing partial protein denaturation, as has been previously reported to occur for rapeseed press cake proteins with similar ultra-fine milling (Rommi et al. 2015b).

6.2.2 CHANGES IN OTHER TECHNO-FUNCTIONAL PROPERTIES

The pasting properties of the cereal-based raw materials and their protein-enriched fractions are largely dependent on the starch content of the sample. Indeed, the higher starch content of barley samples (III) resulted in much higher peak and final viscosities than those observed for wheat and rye bran samples (II). Likewise, the higher starch content of rye compared with wheat bran was noted in the differences in the peak and final viscosities of rye and wheat brans. The positive correlation between the starch content and peak viscosities in viscoamylograms has also been reported for barley fractions by Sumner et al. (1985). In accordance with the observed relationship between the starch content and viscosities, all the fine fractions exhibited lower peak and final viscosities than their raw materials, which delivers enhanced viscosity control during food processing. Understanding the content and quality of starch is important, especially in high-moisture food systems where a heat treatment followed by rapid cooling is usually required, which brings about starch gelatinisation and retrogradation phenomena during processing. In addition to starch content, also the starch granule size is known to play a role in the extent and impact of starch gelatinisation. Kumar and Khatkar (2017) reported lower peak and final viscosities for small B-type starch granules when compared with non-separated wheat starch, including both A- and B-type granules. Similar outcomes were also noted in this work for the protein-enriched wheat bran and barley endosperm fractions. For rye, lower peak viscosity was observed for the protein-enriched fraction, whereas the final viscosity was interestingly higher for the fraction than for the pin disc-milled raw material. Ultra-finely milled rye bran exhibited considerably higher viscosities compared with the pin disc-milled bran. Damaged starch content of the ultra-finely milled bran was higher than that of the pin disc-milled bran, but

typically, damaged starch is known to lower the peak and final viscosities in an RVA, which is contrary to the relationship observed in this work. For example, Hasjim et al. (2013) reported that increasing the damaged starch content in rice flour decreased the final viscosity, and Barrera et al. (2007) showed that an increased amount of damaged starch reduced the peak viscosity of wheat starch suspension. Hence, the reasons for clear differences in the viscosities of the differently milled rye bran samples requires further investigation. In addition to starch, higher IDF content in the brans compared with the protein-enriched fractions may have increased the peak and final viscosities, which is in line with the study by Lai et al. (2011) who reported increased viscosities when adding IDF to rice starch suspensions. Moreover, β-glucan-rich air-classified fractions prepared from oat bran concentrate have been shown to exhibit higher peak and final viscosities in an RVA compared with the raw material (Stevenson et al. 2008).

In high-moisture food systems, the ingredient stability in dispersions has a crucial role. For all the bran materials, decreasing the particle size in both ultra-fine milling and air classification resulted in improvements in the dispersion stability (I, II). Similarly, reduced particle size via microfluidisation has been found to improve the colloidal stability of wheat bran (Rosa-Sibakov et al. 2015b; De Bondt et al. 2020). This is postulated to result from the significant reduction in particle size, which retards sedimentation. The parameters determining dispersion stability are formulated in Stokes' law, which states that the particle size and density, as well as viscosity of the continuous phase affect the sedimentation velocity. Hence, in addition to the reduced particle size, the enrichment of soluble proteins, removal of insoluble carbohydrates and release of some minor grain components during ultra-fine milling of the bran may also have influenced the overall dispersion stability.

In addition to the dispersion stabilities of the bran-water mixtures, considerably improved colloidal stability was noted in emulsification experiments carried out for fine wheat and rye bran samples (II). Understanding the behaviour of the ingredient in an emulsion system offers valuable insights into its application potential, for example, in plant-based milk substitutes. In this work the emulsion stability was followed for 1 d, during which the particle size remained unchanged and only minor clarification was observed on the top of the otherwise homogenous dispersion. In the related literature, Idris et al. (2003) studied the emulsification of wheat bran protein isolate by assessing a 1% protein solution emulsified with 25% oil at neutral pH and observed a decrease in the height of the emulsified layer from 100 to 70% already during three hours. In another study, microscopy observation revealed that wheat bran protein isolate emulsions were inherently unstable (Arte et al. 2019). The emulsification of rice bran protein concentrate (20% oil, 1% protein in an aqueous solution) by Chandi and Sogi (2007) showed that 40% of the total dispersion height was emulsified at pH 7 and the height remained constant during the first day but decreased to approximately 20% in three days. In comparison, in this work, the whole sample was visually

observed to be homogenously emulsified right after emulsification. The soluble components (i.e. proteins and SDF) in the ingredients may have facilitated emulsification in various ways. Some polysaccharides, such as pectin, galactomannan and gum arabic, are known to exhibit interfacial activity, but those were not presumed to be largely present in the studied fractions due to the known biochemical composition of wheat and rye bran DF (Parker et al. 2005). Soluble arabinoxylans, on the other hand, were certainly present and may have contributed to emulsion formation and/or stabilisation by adsorption to the oil–water interface or by increasing the continuous phase viscosity, respectively (Mikkonen et al. 2008). The emulsifying activity and subsequent emulsion stability provided by proteins in food colloids rely on two mechanisms: either the adsorption of soluble molecules to the oil–water interface due to their amphiphilic nature and the formation of a viscoelastic layer or adsorption as colloidal particles, like in the case of Pickering emulsions, providing increased steric stabilisation (Dickinson 2013; McClements 2007). Foaming is also an important parameter to assess when evaluating plant protein functionality and in this work it was studied for the protein-enriched barley fraction (III). The poor foaming capacity of the ingredient can be related to low protein solubility at the studied pH values, as well as to the high content of non-protein components in the fraction. The mechanisms of emulsion or foam formation were not elucidated in the current study, however, they remain highly interesting topics to explore for complex multi-component matrices.

OBC and WBC are relevant properties that provide insight into the ingredient applicability in semi-solid and oil-containing food products since they affect, for example, product texture, stability and mouthfeel (Elleuch et al. 2011; Kinsella 1976). In the current work, the WBC values were in the range of 1.2–2.7 g/g, the lowest values accounting for the only barley sample analysed (i.e. the protein-enriched barley fraction), which contained the lowest amount of DF. The results emphasise the impact of DF components on the WBC of the multicomponent ingredients. In literature, high WBC values have been reported for DF ingredients, such as fine and coarse oat brans (7.0 and 5.7 g/g) (Kurek et al. 2015) and dry-fractionated DF concentrates from rice bran (4.5–4.7 g/g) (Wang et al. 2016). For protein ingredients, the literature values have been shown to range between 1.1. and 5.6 g/g for different rice bran and endosperm, wheat bran and barley protein ingredients (Chandi and Sogi 2007; Idris et al. 2003; Nisov et al. 2020; Wang et al. 2010; C. Wang et al. 2014). Moreover, for wheat bran, values with the opposite impacts of grinding between 2.7 and 4.6 g/g have been reported (Caprez et al. 1986; Zhu et al. 2010). In this work, the fine protein-enriched bran fractions from wheat and rye exhibited low WBC and OBC, and this most probably resulted from the small particle size of the fractions causing lowered physical entrapment of both water and oil to the ingredient matrices (Auffret et al. 1994; De Bondt et al. 2020; Drakos et al. 2017a; Zhang and Moore 1997; Zhu et al. 2010). However, increased surface area after particle size reduction has been

reported to improve the water binding properties in some cases (Elleuch et al. 2011). The significance of the milling intensity applied in ingredient manufacturing was also highlighted. First, higher WBC was observed for the ultra-finely milled rye bran compared with pin disc-milled rye bran. This was suggested to result from the formation of damaged starch, capable of binding more water than native starch (Berton et al. 2002; Drakos et al. 2017a; Niu et al. 2014), during ultra-fine milling of rye bran that has a relatively high starch content. Second, ultra-finely milled wheat bran exhibited lower WBC compared with pin disc-milled wheat bran, which may have resulted from the impact of ultra-fine milling, damaging the physical fibrous bran structures responsible for binding water.

In this work the OBCs ranged between 1.0 and 1.4 g/g. Similar values have been reported in literature, for example, for unground and ground wheat brans (1.2 and 1.6 g/g, respectively; Caprez et al. 1986), wheat bran protein isolate (1.6 g/g; Idris et al. 2003) and rice endosperm protein isolate (1.0 g/g; Nisov et al. 2020). On the contrary, higher values have been reported for dry-fractionated DF concentrates from rice bran (5.1–6.7 g/g, with the initial OBC of the bran being 3.1 g/g; Wang et al. 2016), rice bran protein fractions (1.0–3.6 g/g; C. Wang et al. 2014), rice bran protein concentrates (3.7–9.2 g/g; Chandi and Sogi 2007) and barley protein isolates (5.2–5.7 g/g; Wang et al. 2010).

6.3 MODIFICATION OF THE TECHNO-FUNCTIONAL INGREDIENT PROPERTIES BY ENZYMATIC AND PHYSICAL PROCESSING

The limited food applicability of plant-derived proteins and ingredients may arise from various aspects that decrease their techno-functional properties. In this work, improving the functionality of the protein-enriched rice bran fraction (IV) and barley endosperm fraction (V) in high-moisture food systems was investigated by applying phytase treatment (IV) and ultrasonication with or without pH shifting (V), respectively.

6.3.1 IMPROVING THE HEAT-INDUCED GELATION OF THE PROTEIN-ENRICHED RICE BRAN FRACTION BY PHYTASE TREATMENT

The ability of food ingredients to provide a heat-induced viscosifying or gelling effect is an essential technological functionality that is relevant in a vast range of applications from bakery and dairy to meat and meat analogue products. In this study, the protein-enriched rice bran fraction had a nutritionally interesting composition including a good amount of both protein and soluble DF, and thus, utilisation of 14% solid content provided a protein concentration of 3.3% and total DF content of 3%, enabling the claim that the potential food product is a ‘source of fibre’ according to Regulation (EC) No 1924/2006 (EC 2006).

However, during the air classification-based production process of the protein-enriched fraction from a defatted and pin disc-milled rice bran, the enrichment of phytic acid content to 21.6% also took place. Due to the fact that phytic acid binds minerals and protein, it may reduce their bioavailability and technological functionality (Kies et al. 2006; Rosa-Sibakov et al. 2018; Selle et al. 2000). Hence, the contribution of phytic acid to the heat-induced gelation properties of the protein-enriched rice bran fraction was elucidated using phytase treatment.

At acidic pH below the isoelectric point of most plant proteins, phytic acid and proteins exhibit negative and positive net charges, respectively, resulting in their complexation, which may lower the protein solubility. As expected, phytase treatment of the protein-enriched rice bran fraction improved protein solubility at acidic pH, and similar results are also reported for rice pollards (Kies et al. 2006) and faba bean flour (Rosa-Sibakov et al. 2018). At alkaline pH, both protein and phytic acid exhibit a negative net charge, which results in their dissociation in a liquid environment. Instead of a direct electrostatic interaction at alkaline pH, the presence of a ternary protein-mineral-phytic acid complex has been reported that might affect protein solubilisation as well (Selle et al. 2000). For example, H. Wang et al. (2014) observed increased soy protein solubility at neutral pH after phytic acid addition and postulated it to result from the increased protein net charge due to the ternary complexation. In the current study, the degradation of phytic acid also had a minor negative impact on protein solubility under alkaline conditions.

The heat-induced gelation of the control (non-enzyme treated) protein-enriched rice bran sample resulted in stronger gels, observed as increased G' and WHC, and lowered tan δ values, at alkaline (pH 8) and neutral (pH 6.7) conditions compared with acidic conditions (pH 5). This phenomenon has earlier been reported to also apply in the heat-induced gelation of canola protein isolates (Kim et al. 2016; Yang et al. 2014) and corn germ protein isolates (Sun et al. 2017) and is attributed to increased repulsive interactions at alkaline pH. The fortified repulsive forces retard the formation of large protein aggregates during heating, enabling the formation of small soluble aggregates which are, at the later stages of gelation, responsible for network formation via hydrophobic interactions and hydrogen bonding, as well as disulphide bonding. Due to the fact that the protein solubility values of the control samples differed only a little at pH 6.7 (46%) and pH 8 (49%), the impact arising from protein solubility was not assumed to considerably affect gelation under those pH conditions. Solubility at pH 5 was shown to be remarkably lower for the same protein-enriched rice bran fraction analysed directly as water dispersion when compared with similarly analysed samples at pH 6.7 and 8 (I).

The most significant improvements in the gel strength of the rice bran fraction were proposed to be partially linked to the compounds released concurrently in the degradation of the salt form of phytic acid (phytate), for example, calcium and phosphates, and also with the conformational protein

changes. The protein-related changes were observed as increased surface hydrophobicity after phytase treatment (analysed at pH 6.7), enabling more pronounced hydrophobic protein interactions during heating and cooling and thus aiding gelation. Another important factor facilitating the gelation of the phytase-treated sample at alkaline pH was attributed to the gelation of the pectin present in the fraction. Low methoxyl pectin is known to gel in the presence of calcium via calcium-mediated junction zones and its gelation behaviour has been reported to be the most pronounced at a slightly alkaline pH of 8.5 (Yang et al. 2018). Moreover, the release of phosphates during phytic acid degradation may have improved gelation by reducing the thermal stability of the proteins, as has been shown for lysozyme in the presence of phosphates (Cao et al. 2016). The most significant increases in the G' values during the gelation experiments were observed during the cooling phase. The primary explanation for this lies in the formation of hydrophobic interactions between the proteins that are favoured by cooling (Dickinson 2012), which also further supports the importance of the hydrophobic interactions that improve gelation of the phytase-treated rice bran samples. Moreover, the gelation of pectin mainly occurs during the cooling of a heated dispersion (Urias-Orona et al. 2010; Yang et al. 2018). Other factors that probably affected the structure of the gel system include the insoluble fibres contributing to water retention and the gelatinised and retrograded starch. However, starch concentration remained only at one third of the concentration of protein and DF in the system.

6.3.2 IMPROVING THE PHYSICOCHEMICAL PROPERTIES OF BARLEY PROTEIN INGREDIENTS BY ULTRASOUND TREATMENT AND PH SHIFTING

The protein-enriched barley fraction exhibited limited techno-functional properties. Currently, relatively little is known about the applicability of barley-based ingredients in high-moisture food applications (e.g. dairy substitutes), and therefore, properties relevant in those systems were targeted to be altered. Ultrasound technology in combination with a pH-shifting approach was studied to evaluate the impacts on the techno-functional properties of a protein-enriched barley fraction and a barley protein isolate.

Shifting the pH of both the protein-enriched barley fraction and barley protein isolate dispersions to pH 9 without ultrasound treatment followed by readjustment to pH 7 increased the solubility of both ingredients and also the colloidal stability of the isolate when compared with the samples incubated at only pH 7. A similar impact of alkaline pH shifting on protein solubility has been reported for legume proteins in various studies (Jiang et al. 2018, 2010, 2017; Lee et al. 2016). The protein modifications that occur during the pH-shifting approach have been associated with partial protein unfolding at alkaline pH and then refolding at neutral pH, which leads to the formation of a molten globule state (Christensen and Pain 1991; Hirose 1993; Jiang et al. 2009).

Moreover, the previous research in this field has claimed that pH shifting increases the structural flexibility of proteins and ionic interactions between charged amino acid residues and water. Interestingly, both the previous studies and the current work have revealed that protein solubility did not increase during acidic pH shifting followed by neutralisation, suggesting that for these studied plant proteins, the positive effect only occurs with alkaline shifting.

In this work, the ultrasound treatment of the protein-enriched barley fraction only improved protein solubility significantly at alkaline pH 9, whereas no clear impact was seen in solubility at pH 7 or pH 3. Colloidal stability after ultrasound treatment was improved both at neutral and alkaline pH but not at pH 3, which might be partly related to the effect of neutral and alkaline pH on protein structure and solubility being amplified by cavitation during sonication. For the isolate, significant changes in solubilities were detected as a result of ultrasonication at all the studied pH values and all sonicated samples were also colloidally stable. The difference between the isolate and protein fraction might have derived from the presence of non-protein components that were not considerably affected by the ultrasound treatment applied. However, for both ingredients, a remarkable reduction in particle size was noted after ultrasonication, most probably contributing to the improved dispersion stability (discussed also in Section 6.2.2). Various studies have similarly evidenced ultrasound-assisted improvements in the protein solubility of plant protein isolates and concentrates that mainly originate from legume sources (Hu et al. 2013; Jiang et al. 2017; Martínez-Velasco et al. 2018), as well as millet (Nazari et al. 2018) and canola (Flores-Jiménez et al. 2019), whereas no data is available about the application of ultrasound treatment for improving the solubility of cereal-based hybrid protein ingredients. For both of the barley protein ingredients, a combination of alkaline ultrasound treatment and shifting the pH to 7 improved colloidal stability and protein solubility more than pH shifting or ultrasound treatment (at neutral pH) alone. Similar improvements from the combined ultrasound treatment and pH shifting have been reported for soy protein (Yildiz et al. 2017) and pea protein (Jiang et al. 2017). The combined impact of shifting and sonication was also more pronounced under alkaline rather than under acidic conditions in both the current work and in other research. These findings propose that in order to facilitate the applicability of barley protein ingredients in high-moisture food systems, protein solubility and/or colloidal stability can be improved by both of the studied treatments specifically in the neutral pH region.

No modifications in the SDS-PAGE profiles of either of the barley protein ingredients were observed as a result of ultrasonication. This was in line with most of the literature concerning plant protein functionalisation by ultrasound treatment (Flores-Jiménez et al. 2019; Hu et al. 2013; O'Sullivan et al. 2016, 2015) and depicts that no modifications in primary protein structure took place in sonication but rather, the impacts observed derive from modifications and the breaking of non-covalent interactions modifying the secondary protein

structures (Hu et al. 2015; Jiang et al. 2014; Malik and Saini 2018; Martínez-Velasco et al. 2018) and/or tertiary protein structures (Hu et al. 2015; Xiong et al. 2018). However, it must be noted that contrary data is also available in the literature (Nazari et al. 2018; Resendiz-Vazquez et al. 2017) and, as far as no systematic comparison of different plant ingredients has been made, it remains a matter of discussion whether the impact on the protein profile is species specific or affected by the intensity and conditions of the ultrasound treatment.

6.4 LIMITATIONS OF THE STUDY

6.4.1 EXPERIMENTAL DESIGN

The raw materials studied in the current work were all obtained as samples from industrial-scale mills and, for example, no botanical factors (such as growth conditions, year and varietal purity) were considered. Moreover, due to the variation in grains between different growth locations and years, as well as the specific process modifications in all industrial mills compared to other mills, the milling process for bran removal always results in a somewhat inconsistent composition. Thus, in this work, for example, it remains unknown how much the bran preparation consisted of the whole kernel, which may complicate making general conclusions about the applicability of the studied processes for all possible brans of the same species. Additionally, the amount of non-bran components (i.e. the starchy endosperm and germ) in the bran preparations was not defined.

The dry-fractionated hybrid ingredients are not composed of single, pure components but contain considerable amounts of various biomolecules, all of which affect the behaviour of the ingredient. In regard to characterising the air-classified fractions, the most attention was paid to the protein-enriched fine fractions. However, a more detailed understanding of the properties of the protein-depleted coarse fractions would probably have aided understanding the restrictions in protein enrichment. The potential explanations for the fact that only part of the protein transferred to the protein-enriched fraction are physical entrapment of the protein or aggregation of the protein with the non-proteinaceous particles of the coarse fraction. To clarify this, compositional, techno-functional and microstructural analysis of the coarse fractions would have guided their further processing.

The industrial scalability of the dry fractionation processes is known to be relatively easy. However, in the current study many of the air classifications were performed using maximum or close-to-maximum classifier wheel speeds that are not easily reached in the larger-scale deflector wheel air classifiers applied industrially (Furchner and Zampini 2012). This may present challenges in the potential up-scaling of the processes and lower the industrial relevance of the developed concepts. Nevertheless, one of the barley fractionation cases

(8 000 rpm at pilot scale) was also proven to work well at an industrial scale, as evidenced in Publication V. Another factor possibly limiting industrial-scale processability according to the concepts studied in this work is the SC-CO_2 extraction applied in rice bran defatting. Currently, SC-CO_2 extraction technology is characterised by relatively high investment and operating costs, which limit its industrial use. However, as it is widely accepted that rice bran requires either a stabilisation or oil extraction step to prevent rancidification, various extraction methods are being studied. SC-CO_2 may provide a suitable solution for this pre-treatment step since solvents, such as hexane, which are traditionally applied in oil extraction, are not preferred by the consumers who seek clean label solutions and processing.

6.4.2 ANALYTICS

The selection of the factor for converting the nitrogen content of foods analysed by the Kjeldahl method into protein content is often under debate. In this work, the nitrogen-to-protein conversion factor utilised for rice samples was 5.95 since it is widely applied in the related literature. However, this value was originally determined based on the amino acid composition of rice glutelin (Juliano 1985), which was not assumed to form the major protein class in the bran preparation studied in the current work and therefore shows a potential limitation in the accuracy of the protein contents reported. Similarly, the protein conversion factors applied for the wheat and rye bran materials may raise questions as a factor 6.31 has been recommended for wheat bran and for rye and barley flours the value of 5.83 is typically used. However, since the exact amino acid composition of the raw materials, and especially the fractions, differing considerably from the raw materials, was not possible to be determined within this work, use of the general conversion factor of 6.25 (Jones 1932; Moore et al. 2010) was agreed as emphasised by FAO (2003).

Analysis of DF in this work was carried out according to different methods in Publications I and III when compared to Publication II. In Publications I and III, the older AOAC method 991.43 (AOAC 1995) was utilised, which does not include analysis of the non-digestible oligosaccharides (i.e. soluble DF that stays soluble in ethanol precipitation). On the contrary, the newer AOAC method 2011.25 according to McCleary et al. (2012), which also detects the non-digestible oligosaccharides that stay soluble in ethanol precipitation, was applied in Publication II. Thus, application of the AOAC 2011.25 method for rice (I) and barley (III) ingredients would have presumably resulted in higher SDF values for those ingredients and further increased the SDF:IDF ratios slightly.

6.5 FUTURE PROSPECTS

The development of technologically and nutritionally valuable hybrid ingredients from cereal side streams and their further functionalisation was demonstrated in the current work. Dry fractionation revealed the enrichment of protein in particular but the ratio between DF components was also affected, phytic acid was co-enriched and the impact of fractionation on starch content was shown to be raw-material specific. The more thorough characterisation of the fractions in future studies could reveal other essential aspects relevant for ingredient applicability. For example, the enrichment of antinutrients other than phytic acid (such as trypsin inhibitors) in the fine bran fractions was not studied but may unfortunately limit the food applicability of the produced fractions. The microbiological quality and taste of the ingredients was not within the scope of this work but is highly relevant when considering the real application potential of these ingredients. The technological quality of ingredients is typically studied in model systems as was also adopted in the current study. However, the performance in complex food models or in actual food products, such as plant-based milk substitutes, remains to be studied. The applicability of the hybrid ingredients in solid foods, such as bakery products or dry- and wet-extrudates, should be elucidated in future research since the balanced protein and DF content, combined with the presence of starch and absence of vast amounts of insoluble DF, could offer promising properties for such food models. In regard to functionalisation, solutions for performing modifications for the obtained fractions *in situ* during food manufacturing should be investigated in terms of process feasibility for different food systems. Moreover, functionalisation approaches (i.e. phytase treatment and ultrasonication), which were only applied for selected fractions in this work, would be highly interesting to be researched for the other raw material fractions as well.

Understanding the bioaccessiblity of the proteins in the hybrid ingredients is important, and thus, defining the impact of all the applied processing methods on protein digestibility would yield valuable information in regard to the nutritional quality of the ingredients. First, it would be relevant to understand whether differences in pre-treatments, such as defatting or milling intensity, influence protein digestibility. Moreover, it is important to assess the effect of protein fractionation in air classification on the nutritional quality, especially if the antinutritional factors are enriched in the protein-enriched fractions during their production process. Last, potential modifications to the nutritional quality of the protein-enriched ingredients that occurred during the functionalisation processes (e.g. ultrasound treatment, pH-shifting and phytate degradation) should be elucidated.

Protein enrichment from the pre-treated cereal side streams by milling and air classification was shown to be successful, whereas the mass yields and protein separation efficiencies can be considered limiting factors in terms of process feasibility. In order to understand the structural constraints of the raw

materials that restrict component fractionation, more sophisticated imaging techniques, combined with an increased understanding of the mechanical and biochemical raw material structures, could allow improving the processes and recovering more of the desired components in fractionation. Even though the dry-fractionated ingredients are expected to, for example, effectively retain the native techno-functional properties of the ingredient components, vitamins and minerals, a side-stream fraction is always generated in fractionation. In many cases, attention is only paid to the fraction enriched in the most desired components (protein and DF in this work), whereas the applicability, properties and composition of the side-stream fraction is less studied. Similarly, in this work, the coarse, protein-depleted fractions were not studied despite the fact that they are projected to contribute significantly to the overall feasibility of the developed processing concepts. In the future, it would be important to identify potential uses for the coarse, protein-depleted fractions. Such uses could include their application for fibre enrichment purposes in food, as feedstock in single-cell protein production or non-food purposes, such as biodegradable packaging or animal feed.

Sustainability and resource sufficiency are key aspects of the newly developed food processing concepts. Despite the gentle and low-energy-consuming nature of the dry fractionation routes, improving the commercial milling approaches that are closely linked to the processes studied in this work should be targeted. From an industrial point of view, the modification of the current production processes that yield refined flour, in order to directly recover more of the valuable side stream components as food raw materials, would improve the raw material utilisation. More specifically, the fractionation, collection and defined applicability of all the grain layers (pericarp, aleurone, subaleurone, the rest of the starchy endosperm) might provide added value and reduce the production of side streams. Preliminary techno-economic assessment of protein enrichment from wheat bran has previously been carried out and proven feasible (Hytönen and Sorsamäki 2018). However, in order to justify the use of the other developed dry fractionation concepts for protein enrichment from rice and rye brans, and the barley endosperm fraction, the feasibility of these processes should also be considered.

7 CONCLUSIONS

The increased consumption of plant-based ingredients and the valorisation of agricultural side streams for food are supported by nutritional, environmental and food security-related factors. Currently, most of the plant ingredients, especially plant proteins, are produced via water-intensive production processes in which severe treatment conditions may negatively affect the important ingredient properties, such as nutritional quality and technological functionality. On the contrary, dry fractionation is regarded more sustainable and gentle processing approach allowing mild refinement of the plant raw materials into ingredients enriched with desired components. Instead of pure component isolation, dry fractionation yields in multicomponent, hybrid ingredient fractions in which the technological and nutritional properties of all the components, such as protein, DF and starch, may provide multiple benefits.

In this work, use of dry fractionation for the production of hybrid ingredients from cereal side streams was facilitated by pre-treatments that targeted removing the main constraints of each ingredient. Rice bran with an initial oil content of 22% was extracted with SC-CO_2 for defatting, which allowed further processing of the bran by pin disc milling and air classification. A one-step air classification process increased the protein content from 18.5% in the defatted rice bran to 25.7%, with 27.2% mass yield and 38.0% PSE, from the raw material. Alternatively, the air classification of the defatted bran without a pre-milling step allowed the removal of pericarp particles from the fine fraction, and further milling and air classification of the coarse fraction resulted in even higher protein content (27.4%). For wheat and rye brans, the known resistance of aleurone cell wall structures to dry milling was minimised by including a pre-drying step (48 h at 40°C). This enhanced particle size reduction by pin disc milling, increased the mass yields of the protein-enriched fractions in air classification and allowed protein enrichment from 16.4 to 30.9% for wheat bran and from 14.7 to 30.7% for rye bran. Regarding the barley endosperm fraction, the cohesiveness of the original flour was overcome by mixing the material with a flowability aid, which increased the protein content (up to 28.3%), mass yield and PSE in air classification, presumably due to improved material dispersability in the air during fractionation.

The dry-fractionated protein-enriched hybrid ingredients prepared from the bran raw materials exhibited higher soluble-to-insoluble DF ratios compared with their raw materials, suggesting the removal of insoluble pericarp structures in fractionation. Moreover, a considerable increase in the phytic acid content of the bran ingredients was observed in fractionation, revealing the enrichment of aleurone-origin proteins. Species-specific differences in the fractionation behaviour of starch in the three studied brans were detected. Air classification allowed rather efficient removal of starch from the protein-

enriched rice bran fraction, presumably due to unaffected starch amyloplast structures in fat extraction and milling. The starch content of wheat and rye brans, containing both small and large starch granules from the starchy endosperm, was not affected by fractionation. The fourth raw material studied in this work was a high-starch barley endosperm fraction. Microstructural observations of the air-classified fractions produced from this raw material revealed that, despite reduced starch content in air classification, further protein enrichment was restricted by the location of small starch granules tightly embedded in a storage protein matrix in small-sized particles.

Dry fractionation altered the technological functionalities of the raw material cereal side streams (protein solubility, dispersion stability, water and oil binding capacities, pasting properties) and the observed modifications proposed the enhanced applicability of the protein-enriched fractions in high-moisture food matrices. Protein-enriched fractions from rice and wheat brans showed higher protein solubility values in water compared with their raw materials, especially at neutral and alkaline conditions. Increased protein solubility, together with the increase in phytic acid content and protein profile analysis, indicates the enrichment of the water- and salt-soluble aleurone proteins during air classification. The protein-enriched rye bran fraction exhibited lower solubility all through the studied pH conditions, which may result from the enrichment of proteins complexed with other macro-/micromolecules or more insoluble subaleurone and other starchy endosperm proteins in the protein-enriched fractions, which requires further assessment. Dry fractionation also altered the pasting properties of the ingredients, and for example, the lowered peak and final viscosities in heating and cooling may alleviate the challenges related to the processability of high-starch ingredients. WBC and OBC were lower for the fractions compared with the raw materials, which may deliver advantages in some food applications. In comparison, ultra-fine milling, which was studied as a means for reducing the particle size of wheat and rye brans to close to that of the air-classified ingredients, lowered protein solubility and increased pasting viscosities as well as WBCs and OBCs when compared with the air-classified fractions. However, the ultra-finely milled brans did not differ from the air-classified protein-enriched fractions in terms of emulsification ability.

The protein-enriched barley ingredient showed limited functionalities but was considerably modified by ultrasound treatment with and without pH shifting. Impact of ultrasonication depended on the treatment pH and showed the most promising alterations at alkaline pH. Further modifications were observed as a result of pH shifting, especially to alkaline conditions and back to neutral. The improvements in technological functionality were detected as increased protein solubility and colloidal stability, which are considered relevant properties in high-moisture food systems. In addition to ultrasound treatment, the functionalisation of the protein- and phytic acid-enriched rice bran fraction using phytase was studied. The heat-induced gelation properties of the ingredient under alkaline conditions were considerably improved as a

result of phytic acid degradation and the effect was presumed to primarily result from increased protein surface hydrophobicity facilitating the gelation and gelling of pectin due to the calcium ions released from the phytate complex.

This study increased understanding of the factors affecting dry fractionation of different cereal side streams aiming at the production of protein-enriched hybrid ingredients. Selected pre-treatments were evidenced to improve the behaviour of the material in dry fractionation and the dry-fractionated ingredients exhibited modified techno-functional properties when compared with their raw materials. Bioprocessing of the protein-enriched rice bran fraction using phytase and ultrasound- and pH-shifting-aided processing of the protein-enriched barley fraction were effective tools in further improving the technological quality of the ingredients. The results propose that side stream valorisation via dry fractionation alone or combined with subsequent targeted functionalisation approaches increases both the nutritional and techno-functional properties of the ingredients and open new possibilities for the food applicability of the undervalued cereal side streams.

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